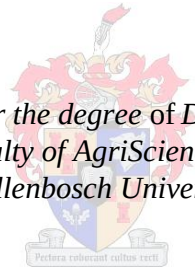
Population genetic structure and abundance of two *Ceratitis* species (Tephritidae) of agricultural importance in South Africa

by
Minette Karsten



Dissertation presented for the degree of *Doctor of Philosophy in the Faculty of AgriSciences at Stellenbosch University*

Promotor: Dr. Pia Addison
Co-promotor: Prof. John S. Terblanche
Co-promotor: Prof. Bettine Jansen van Vuuren

December 2014

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2014

ABSTRACT

Research in the field of biological invasions has increased dramatically in the last two decades, especially due to the impact of human activity such as, transport, travel and international trade. Different stages of invasion have been proposed, each stage posing different barriers that must be overcome by the organism for it to become established. For the prevention of new invasions and the formulation of a successful integrated pest management program (IPM), knowledge of natural species community assemblage, as well as movement patterns, temporal distribution and invasion pathways are critical. In this dissertation I make use of two agricultural important fruit fly species, *Ceratitis capitata* amd *C. rosa*, in the Tephritidae family in different stages of the invasion process using different research methods to (i) investigate seasonal variation in fruit fly abundance in orchards and natural vegetation in the Western Cape to determine whether natural vegetation is used as possible refugia; (ii) to investigate maccrogeographic population structure of *C. capitata* with a focus on southern Africa to reconstruct and test *C. capitata's* invasion pathway using a Bayesian framework; (iii) to investigate the population genetic structure, using molecular and morphological markers, to estimate gene flow and dispersal ability of *C. rosa* in South Africa. Results showed that *C. capitata* was the most abundant species captured and Biolure® the most effective lure. The largest number of individuals of either species (*C. rosa* and *C. capitata*) were captured in austral autumn (March-May) in both orchards and natural habitat with low capture rates throughout the rest of the year. Orchards and natural habitats were significantly different with higher trap catches always found in orchards. Based on microsatellite markers, *C. capitata* showed a decrease in genetic diversity moving away from the native range (Africa) into the introduced range (Australia, Greece, Guatemala, Madeira). Moreover, there was a clear pattern of differentiation between the African continent and the rest of the world indicating low levels of genetic connectivity. High connectivity throughout the African continent is problematic as this suggests that new invasions will move and colonize new areas unimpeded after first introduction. Lastly, my results indicate that there is no population structure in *C. rosa* within South Africa. This indicates that there are high levels of connectivity between different pest-occupied sites within the country and suggests that area-wide pest management should be undertaken on a much larger, preferably country-wide, scale. My results are discussed in the framework of invasion biology as well as integrated pest management. In conclusion, when investigating biological invasions, information from organismal biology and ecology as well as molecular biology can be valuable to inform decision-making regarding prevention and mitigation of pest species.

OPSOMMING

Navorsing op die gebied van indringer biologie het in die laaste twee dekades dramaties toegeneem, veral ten opsigte van die impak van menslike aktiwiteite soos vervoer, reis en internasionale handel op nuwe kolonisasies. Verskillende fases is voorgestel as deel van die indringingsproses en vir ‘n spesie om suksesvol te kan vestig moet die organisme die verskillende hindernisse in elke fase oorkom. Kennis van die natuurlike samestelling van spesies-gemeenskappe, spesie bewegingspatrone, temporele verspreiding en roetes van indringing is van kritieke belang vir die voorkoming van nuwe indringers sowel as die formulering van 'n suksesvolle geïntegreerde plaagbeheerprogram (GPB). In hierdie verhandeling pas ek verskillende navorsingsmetodes toe op twee vrugtevlieg spesies, *Ceratitis capitata* en *C. rosa*, van belang vir die landbou, in die Tephritidae familie in verskillende stadiums van die indringingsproses om (i) die seisoenale variasie in vrugtevlieg getalle vas te stel in vrugteboorde en natuurlike habitate in die Wes-Kaap en om te bepaal of die natuurlike habitat gebruik word as refugia; (ii) die wêreldwye bevolking genetiese struktuur van *Ceratitis capitata* te ondersoek met ‘n fokus op suidelike Afrika sowel as die indringing te rekonstrueer en te toets deur gebruik te maak van ‘n Bayesian raamwerk; (iii) die bevolking genetiese struktuur van *Ceratitis rosa* in Suid-Afrika te ondersoek met behulp van molekulêre en morfologiese merkers. Resultate het getoon dat *C. capitata* die mees algemene spesies was en dat Biolure® die mees doeltreffende lokmiddel was. Die grootste aantal individue van beide spesies (*C. rosa* en *C. capitata*) is gevang in die suidelike halfrond herfs (Maart-Mei) met lae vangste die res van die jaar. Boorde was beduidend anders as die natuurlike habitat, altyd met hoër vangste. Gebaseer op mikrosatelliet merkers het *C. capitata* 'n verlaging in genetiese diversiteit getoon vanaf die inheemse gebied (Afrika) na nuut gekolaniseerde gebiede (Australië, Griekeland, Guatemala, Madeira). Verder was daar 'n duidelike genetiese onderskeid tussen die Afrika-kontinent en die res van die wêreld. Die hoë vlak van verbinding op die Afrika-vasteland kan problematies wees omdat dit daarop dui dat nuwe besettings onbelemmerd na nuwe gebiede sal beweeg na die eerste kolonisasie. Laastens dui my resultate dat daar geen bevolking genetiese struktuur in *C. rosa* in Suid-Afrika is nie. Dit dui daarop dat daar verbindings is tussen verskillende plaag-besette lokaliteite in die land en dat area-wye plaag beheer op 'n veel groter, verkieslik landwye skaal onderneem moet word. Ek bespreek my resultate in die raamwerk van indringer biologie asook geïntegreerde plaagbeheer. Ten slotte, wanneer indringer biologie ondersoek word, is inligting met betrekking tot die organisme se biologie en ekologie sowel as molekulêre biologie van uiterse belang in die besluitnemingsproses om lig te werp op die voorkoming en beheer van peste.

ACKNOWLEDGEMENTS

Research conducted for the completion of this dissertation was funded by the Human Resources Programme (THRIP) from the National Research Foundation of South Africa [Grant specific unique reference number (UID) 71909] and in part by the National Research Foundation of South Africa, the South African Apple and Pear Producers Association (SAAPPA), the South African Stone Fruit Producers Association (SASPA), as well as the South African Table Grape Industry (SATGI). The Grantholder acknowledges that opinions, findings and conclusions or recommendations expressed in any publication generated by NRF supported research are that of the author(s), and that the NRF accepts no liability whatsoever in this regard. I am very grateful for financial support during my PhD from a NRF Innovation Doctoral scholarship as well as a Stellenbosch University Postgraduate Merit Bursary.

This dissertation was made possible through the help of a whole host of people. First and foremost I would like to sincerely thank the promotors of my dissertation Dr. Pia Addison, Prof. John Terblanche and Prof. Bettine Jansen van Vuuren for their tireless support, guidance and help. John, thank you for cheering me on and all the while encouraging me to write better and make it a sexy story. Pia, thank you for introducing me to the world of fruit flies and sharing my "they're so pretty" moments. Bettine, thank you for improving my writing and sharing your genetic knowledge.

I am grateful to farmers in South Africa for access to their farms and sample collection, as well as Marelize de Villiers, Luís Dantas, Walther Enkerlin, Ernie Steiner, Sunday Ekesi, Luis Bota, Louisa Makumbe, Maulid Mwatawala, Nikos Papadopoulos and Ibrahim Solo for samples. I would also like to thank C. Vorster for assisting me with fieldwork, driving around when we just can't find that trap and running from baboons in Franschhoek. Tessa Cooper and Elani Steenkamp for helping me count thousands of flies, Francois Bekker for taking photos of my "pretty" wings and Adrienne Leussa for endlessly packing tips in the Molecular lab. Thank you. I would also like to thank Ruan Veldtman who provided valuable advice on generalized linear mixed models as well as Chris Klingenberg and Hugo Benítez for help with geometric morphometrics. I am grateful for discussions and constructive comments of Chapter 4 by Massi Virgilio and Marc de Meyer. Microsatellite data were genotyped at the Central Analytical Facility, Stellenbosch University.

Thanks to all my APE and IPM labmates for help and advice with labwork, maps (Matt Hill) and keeping me sane. Thanks for putting up with smelly traps, occupation of lab computers to run simulations and telling me it's almost over.

A special thanks to my friends, my family, my parents, Madene and Johannes for love and support. Thank you for believing in my abilities, cheering me on and teasing me that all I do is catch bugs for a living.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: GENERAL INTRODUCTION

Research in the field of biological invasions has increased dramatically in the last two decades, especially research on the impact of human activity such as, transport, travel and international trade on new invasions (Sakai *et al*. 2001; Perrings *et al*. 2005; Meyerson & Mooney 2007; Hulme 2009). In the future, biological invasions are expected to become an even bigger problem especially in the light of rising population numbers placing increased pressure on food security (Godfray *et al*. 2010). These biological invasions are one of the most important driving forces behind degrading habitats, influencing native biodiversity and changing ecosystem functioning (Le Roux & Wieczorek 2009; Wagner & Van Driesche 2010). Predicting what species will become invasive has been an urgent and longstanding priority in invasion biology (e.g. Kimberling 2004; Van Kleunen *et al*. 2010; Higgins & Richardson 2014) together with improved predictive models based on adequate biological data for well-developed control methods (Kirk *et al*. 2013). Much of what is known about biological invasions derives from studies in plant invasions (e.g. Bosssdorf *et al*. 2005; Van Kleunen *et al*. 2010). There are many factors that have been suggested as contributing to invasive success. Three of these are especially important in plants and possibly also in invertebrate pests. The first is high genetic diversity associated with the potential for adaptation to the novel habitat (Schierenbeck & Ellstrand 2009), the second is preadaptation or phenotypic plasticity (Van Kleunen *et al*. 2010) and finally some similarity between the native and novel habitat (Hayes & Barry 2008).

In a recent study, Blackburn *et al*. (2011) proposed an integrated framework for the invasion process, in which they suggest different invasion stages, each stage with its own set of barriers that need to be overcome by the organism in order for the species to become invasive (Fig 1.1). The first stage is transport, with the second being introduction into a new geographical location. Invertebrate pests overcome this first geographic barrier as they are moved around the world via many human-mediated pathways (Hulme 2009) which include (but are not limited to) human travel as well as the trade of commodities by air and sea. In these first two stages management mainly consists of preventing new invasions into an uninvaded location. Species that are abundant and widespread are more likely to be transported outside of their native range than rarer species with narrower distributions (Blackburn *et al*. 2009). This type of "sub-sampling" from the native range leads to small numbers of individuals introduced at a specific time point. Establishment success is closely linked to propagule pressure, which includes the number and frequency of individuals introduced (Lockwood *et al*. 2009), and has been shown to be one of the most important factors in determining the initial colonization success of a species and not necessarily species-

level characteristics such as net reproduction or development rates (Lockwood *et al*. 2005; Lockwood *et al*. 2009; Simberloff 2009a; Blackburn *et al*. 2013). The larger the propagule size the more likely the species is to establish in the novel habitat. On the other hand Szűcs *et al*. (2014) showed that although propagule pressure is important in the initial establishment stage, genetic processes are important for subsequent spread and growth of the population. After this initial introduction to a novel location, the species faces many additional challenges before successfully establishing (Blackburn *et al*. 2011). The next stage is that of establishment and the final stage is that of spread. In these latter two stages, management efforts include eradication, containment and mitigation. In the final stage the new invasive species are established in the new geographic location. The intuitive next questions are then "where did it come from" (invasion pathway) in combination with "how has it moved throughout the new geographical range" (population structure)? Barclay & colleagues (2011) developed a conceptual model to determine the minimum size for an area-wide integrated pest management strategy to be technically viable and also economically acceptable. They highlight the importance of basic biological knowledge, knowledge about population structure and the movement of individuals between pest-occupied sites to ensure effective and successful control measures.

The role of genetics in understanding and preventing biological invasions

After a species establishes in a novel environment as well as in its subsequent range expansion it undergoes an array of genetic changes. Therefore it is important to understand changes in genetic diversity as a symptom of these evolutionary changes which can potentially assist in predicting whether species will be able to counter management practices through evolution, specifically in the light of resistance against pesticides or biological control agents (Barrett 1992). A newly established population can have diminished genetic variation due to a small number of founders from the same source population which can lead to inbreeding depression (Nieminen *et al*. 2001; Sakai *et al*. 2001) or a lack of evolutionary potential.

Different molecular markers including protein and DNA genetic markers are widely applied to investigate a variety of questions important for biological invasions. These include: pest species identification, identifying mechanisms in resistance to pesticides and assessing whether pest management practises are effective. Moreover neutral markers are especially useful for the reconstruction of invasion pathways and routes of colonisation in combination with other demographic processes such as dispersal patterns and population bottlenecks

(Fitzpatrick *et al*. 2012; Kirk *et al*. 2013). Here I will focus on the use of population genetics in assessing management practices, investigating demography (especially dispersal) and reconstructing invasion pathways.

Data from population genetic studies have been shown to, to some extent, be useful in measuring and predicting the outcome of different management practices (Mazzi & Dorn 2012). Measures of effective population size (N_E) and movement (gene flow) between sites under different types of management schemes can aid in choosing the most successful method for the specific pest (Kirk *et al*. 2013). Additionally, genetic tools can help distinguish between endemism and outbreak in an area and allow for the inference of the source of the invasion (Malacrida *et al*. 2007).

Understanding dispersal of organisms as well as their demography can help identify new invasions, help facilitate monitoring of already established species in a region and aid the prevention of new introductions (Estoup & Guilemaud 2010; Kirk *et al*. 2013). For example, identifying the native range of a species can assist in finding natural enemies to be used as biological control agents as part of an integrated pest management program (IPM). Most commonly microsatellites or amplified fragment length polymorphism markers (AFLP) are employed in the reconstruction of invasion pathways, the quantification of gene flow (dispersal) across multiple spatial scales, identification of migrants and the estimation of admixture (Handley *et al*. 2011; Kirk *et al*. 2013). Furthermore, gaining information on similar topics within a country or region can be useful to develop management strategies. Dispersal is an important consideration in successful invasive species but is notoriously difficult to measure in the field. Dispersal allows organisms to move to new suitable habitat (Broquet & Petit 2009) specifically in the light of climate change (Kokko & López-Sepulcre 2006). The dispersal of insects has in the past been predominantly investigated using mark-and-recapture methods (e.g. fluorescent dust) as well as (less often used) radar (e.g. harmonic radar) often resulting in biased estimates, because sampling may be restricted in space and time (Koenig *et al*. 1996). In contrast, indirect approaches, for example, using the genetic structure of a species are averaged over many generations and the use of molecular tools in the investigation of movement of individuals have proven invaluable (Bohonak 1999). Controversy exists as the question remains whether measures of dispersal from direct and indirect methods can be directly compared, with some studies finding agreement (Peterson & Denno 1998; Bohonak 1999) and others showing that genetic tools estimate higher levels of dispersal than direct methods (Kourti 2004). Reasons for these discrepancies include small

sample sizes, migration rates not being constant and individuals migrating into new populations not successfully producing off-spring (Caccone 1985; Caccone & Sbordoni 1987; Grant & Little 1992).

The worldwide invasion of economic important fruit flies

Fruit flies in the Tephritidae family are some of the most significant agricultural pests worldwide, constituting more than 4000 species (White & Elson-Harris 1994). Many of the species in this family have an Afrotropical (De Meyer 2000; De Meyer 2005; Barr & McPheron 2006) or Asian origin (Drew *et al*. 2005) but have subsequently spread to locations outside of their natural ranges and some have become highly invasive in their new introduced range and offer a diverse set of invasion case histories. Their high invasion capacity is most likely due to their broad larval host range (McPheron & Steck 1996), high rates of reproduction, broad environmental niches (Duyck *et al*. 2006) and the increase in passive dispersion due to global trade and tourism (Villablanca *et al*. 1998; Liebhold *et al*. 2006; Hulme 2009). Many of these aforementioned traits however differ between different fruit fly species and this makes predicting invasion potential for the different species unfeasible.

The most notorious and widespread fruit fly worldwide is perhaps *Ceratitis capitata* (Weidemann). From its Afrotropical native range (De Meyer *et al*. 2002) *C. capitata* colonised the Mediterranean Basin, likely human-mediated (Maddison & Bartlett 1989), consequently spreading to the rest of the Mediterranean region (Fimiani 1989) with drift as well as possibly selection playing a role (Malacrida *et al*. 2007). This method of spread is supported by population genetic structure studies in which different populations in the region shows a correlation between genetic and geographic distance indicating graded movement along the basin (Malacrida *et al*. 1998; Gomulski *et al*. 1998). This first wave of *C. capitata* introductions were followed by a secondary introduction of Australia from these European introductions in the 1890's (Hooper & Drew 1989; Vera *et al*. 2002; Bonizzoni *et al*. 2004). The next leg of introductions in *C. capitata's* global colonization was into Latin America and the Pacific which are largely attributed to the establishment of additional trade routes and an increased ease of human travel (Malacrida *et al*. 1998; Bonizzoni *et al*. 2004). From the Americas *C. capitata* was first reported from Costa Rica (1955) (Enkerlin *et al*. 1989) from which it spread south to Guatemala (1976) and along the coffee belt to Mexico (Harris 1989). This expansion of the species range was probably aided by multiple introductions as well as increased genetic diversity either by admixture or hybridisation between established populations (Malacrida *et al*. 2007). After the introduction of *C. capitata* to Hawaii (1910) it

had been replaced by *Bactrocera dorsalis* (Hendel) (1945) (Duyck *et al*. 2004). Moreover, *C. capitata* has colonized the Indian Ocean Islands of La Réunion (1939) and Mauritius (1942) (Orian & Moutia 1960) and subsequently displaced *C. catoirii* Guérin-Mèneville which was indigenous on both islands.

Two other members of the genus *Ceratitis*, *C. rosa* Karsh and *C. fasciventris* Bezzi, are of major invasion concern because of evidence for large reservoirs of genetic diversity in these species (Baliraine *et al*. 2004; Virgilio *et al*. 2008; Virgilio *et al*. 2013). *Ceratitis rosa* has already invaded Mauritius (1953) and La Réunion (1955) (White & Elson-Harris 1994; De Meyer 2001) and largely outcompeted *C. capitata* (Duyck & Quilici 2002). Both these islands have also been invaded by *Bactrocera zonata* (Saunders), Mauritius in 1987 and La Réunion in 1991 (White *et al*. 2001). *Ceratitis fasciventris* has not yet moved outside of the African continent, but it is expanding its range from Kenya into Uganda which is maintained by continuous gene flow, possibly aided by trade between these two countries (Baliraine *et al*. 2004).

Another genus containing many highly invasive fruit flies is *Bactrocera* Macquart consisting of about 440 species (White & Elson-Harris 1994) which are mostly confined to the Oriental and Australia (Clarke *et al*. 2005; Malacrida *et al*. 2007). Of these *Bactrocera tryoni* (Froggatt), members of the *Bactrocera dorsalis* complex (*B. dorsalis* (Hendel), *B. carambolae* Drew & Hancock, *B. philippinensis* Drew & Hancock and *B. papayae* Drew & Hancock), *Bactrocera cucurbitae* (Coquillett) and *Bactrocera oleae* (Rossi) are probably the most well-known invasives worldwide (Clarke *et al*. 2005).

Bactrocera tryoni is thought to be native to the tropical and subtropical areas of coastal Queensland and northern New South Wales (Gilchrist *et al*. 2006), but has adapted to many cultivated host fruit and spread to eastern Australia, subsequently invading French Polynesia, New Caledonia, Pitcairn Islands and Cook Islands (White & Elson-Harris 1994; Clarke *et al*. 2011). It is now on occasion found in south-western New South Wales, inland Victoria, South Australia and Western Australia (Yu *et al*. 2001; Gilchrist 2004; Gilchrist *et al*. 2006; Clarke *et al*. 2011).

Bactrocera dorsalis was first recorded in Taiwan in 1912 (Drew & Hancock 1994) and thereafter found in many countries throughout the Asia-Pacific including China all the way to the Indian subcontinent (Aketarawong *et al*. 2007). Outside of this area (Asia) *B. dorsalis* have been found in Hawaii (1945), Guam (1947) and occasionally in California and Florida

(1960-1990) (White & Elson-Harris 1994; Aketarawong *et al*. 2007, 2014). In 2003 a member of the *B. dorsalis* complex was found in Kenya (Lux *et al*. 2003) and described as a new species *B. invadens* Drew, Tsuruta & White (Drew *et al*. 2005), this species is now believed to be synonymous to *B. dorsalis*. After its establishment on the African continent *B. dorsalis* (*B. invadens*) has spread too many eastern, western and central African countries (Mwatawala *et al*. 2004; Drew *et al*. 2005) and is now distributed throughout tropical Africa from Senegal to Mozambique and also the Comoro Islands (De Meyer *et al*. 2010). More recently (2010) it has also been found in the northern parts of South Africa (Manrakhan *et al*. 2011) and after eradication attempts have now been declared to be present in the north although the rest of the country remains pest free. From many countries in which *B. dorsalis* had established initially it has now been eradicated including Ryukyu Islands in Japan, Nauru, Guam and Northern Mariana Islands (Stephens *et al*. 2007). *B. carambolae* is native to Indonesia, Malaysia and Southern Thailand and has invaded parts of South America including Suriname (around 1975) (Van Sauers-Muller 1991) as well as Guyana (eradicated), French Guiana, Amapa and northern Brazil (Drew & Hancock 1994). *Bactrocera philippinensis* occupy eastern parts of south-east Asia including the Philippines and Borneo and has been detected in Australia although subsequently eradicated (Smith *et al*. 2000). The last member of the complex of significance is *B. papayae* which are native to Malaysia, parts of Thailand as well as throughout western Indonesia (Drew & Hancock 1994). This species has been introduced to Irian Jaya after which it was detected on the borders of Papua New Guinea and subsequently spread throughout the country (Sar *et al*. 2001). In 1993 this species was also detected in Torres Strait, Queensland (Fay *et al*. 1997) after which it was swiftly eradicated with a second incursion in the Cairns-Mareeba-Mossman region where it was eradicated, although occasional outbreaks persist (Cantrell *et al*. 2002).

Bactrocera cucurbitae possibly has an Indian origin (Bezzi 1913) but has spread to Oceania (including the Mariana Islands and New Guinea), Hawaii and East Asia (including Pakistan, India, Bangladesh, Nepal, China, Indonesia and Philippines) (Bess *et al*. 1961). It has also invaded the African continent, Tanzania in 1936, West Africa around 1999 (Vayssières *et al*. 2008) as well as Mauritius (1942) and La Réunion (1972) (Oke 2008).

Bactrocera oleae's distribution is linked to cultivated olive trees and this species with expanding olive cultivation worldwide (Katsoyannos 1992; Augustinos *et al*. 2002). Its native range is believed to be African and it is now found throughout the Mediterranean basin, the

Middle East, Oriental Asia (White & Elson-Harris 1994) as well as Mexico and California (Rice 2000).

Anastrepha suspensa was restricted to the Bahamas and Greater Antilles until the 1960s (Weems *et al*. 2001), but has subsequently spread to the West Indies and nearby islands (Cuba, Jamaica, Hispaniola, Puerto Rico) as well as southern Florida (White & Elson-Harris 1994).

The use of population genetics in fruit fly invasions

Population genetics have been shown to be useful to investigate the colonization process, routes of invasion and the underlying evolutionary forces that shape population structure in an array of tephritids (e.g. Bonizzoni *et al*. 2001, 2004; Gilchrist & Meats 2009; Aketarawong *et al*. 2014). Here I will focus on some examples of those species mentioned in the "***The worldwide invasion of economic important fruit flies***" section of this chapter.

A lot of what we know about tephritid invasion genetics is based on studies from *Ceratitis capitata*. Earlier studies of population genetic structure of *C. capitata* was performed using a variety of genetic markers including: enzyme electrophoresis (Gasperi *et al*. 1991; Kourti 2004), random amplified polymorphic DNA (RAPD) (Baruffi *et al*. 1995), multilocus enzyme electrophoresis (Baruffi *et al*. 1995; Malacrida *et al*. 1998), polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) (Barr 2009), sequence data from nuclear introns (Gomulski *et al*. 1998) and mitochondrial markers (Meixner *et al*. 2002). Information from all of these markers indicated genetic differences between the native populations of East Africa and adventive populations sampled elsewhere in the world (Mediterranean, Pacific and Americas). Studies based on larger geographical scales indicated that *C. capitata* populations showed decreased genetic variability at two scales from Africa to the Mediterranean Basin as well as from Latin America to the Pacific (Gomulski *et al*. 1998; Malacrida *et al*. 1998; Gasperi *et al*. 2002). There are also cases with clear isolation-by-distance patterns and some in which ancestral variability has remained present. These patterns are characteristic of multiple introductions or recent range expansion into an area (Gasperi *et al*. 2002; Malacrida *et al*. 2007). Studies employing microsatellites to investigate population structure in *C. capitata* are numerous. Bonizzoni *et al*. (2000) developed 43 microsatellite markers and characterised ten of these for six geographic locations (Kenya, La Réunion, Madeira, South Italy, Greece and Peru). They showed the same break in variation at the two scales identified from earlier studies. These same ten microsatellites was used in a subsequent

study focussing on relationships between different *C. capitata* populations in California as well as their potential connection to populations in Hawaii and Latin America (Guatemala, El Salvador, Ecuador, Brazil, Argentina and Peru) (Bonizzoni *et al*. 2001). They showed that Californian flies are closely related to flies from Guatemala, populations from California and San Diego group together and that flies captured in the Los Angeles Basin throughout a period of time (1992-1997) are genetically similar. Baliraine *et al*. (2003) showed that the same set of microsatellite markers could be used to investigate *C. capitata*, *C. rosa*, *C. fasciventris* and *C. cosyra* (Walker). Bonizzoni *et al*. (2004) investigated the genetic structure of *C. capitata* in Australia as well as the invasion history and origin of outbreaks. They show that *C. capitata* in Australia probably derived from the Mediterranean, that the Perth area (Western Australia) acts as a source area for other regions in Australia and that genetic variation is distributed homogeneously within Australia in combination with a lack of population differentiation in the coastal regions of Australia from Perth northwards. Malacrida *et al*. (2007) reviewed the invasion history of *C. capitata* based on molecular markers and showed that there is clear evidence for invasion of the Mediterranean Basin from Africa (Kenya) and subsequent invasions to Latin America and the Pacific. Alaoui *et al*. (2010) showed population genetic structure in Morocco for *C. capitata* populations in endemic Argan forests, likely driven by altitudinal differences between populations. Karsten *et al*. (2013) showed high levels of genetic diversity throughout South African *C. capitata* populations as well as a lack of population structure.

Virgilio *et al*. (2008) investigated the phylogenetic relationships between *C. rosa*, *C. fasciventris* and *C. anonae* Graham using mitochondrial (16S, COI, ND6) and nuclear markers (period, ITS1) throughout their African distribution including La Réunion. The different markers used in this study could not recover the three species as separate clades but divided *C. fasciventris* into two separate clades (East and West African). Using microsatellites Baliraine *et al*. (2004) investigated levels of genetic variation as well as genetic differentiation in *C. rosa* and *C. fasciventris*. They showed that higher levels of genetic diversity exsist in mainland populations versus those on islands (Mauritius and La Réunion) as well as lower levels of differentiation across the African mainland than for the island populations of *C. rosa*. In *C. fasciventris* they showed no differentiation between populations in Uganda and Kenya indicating high levels of gene flow between the two countries. Virgilio *et al*. (2013) identified two genetic clusters in *C. rosa* (R1 and R2) as well as two within *C. fasciventris* (F1 and F2). They also show no hierarchical structured populations as well as an indication of gene flow within these identified genetic clusters.

In ancestral regions of Australia, Yu *et al*. (2001) investigated the population structure of *B. tryoni*. They showed that these populations could divivded into three groups. Subsequently Gilchrist *et al*. (2006) investigated population structure across the ecological range of *B. tryoni* in Australia. They showed that in samples from the ancestral area there were high levels of gene flow and in the temperate region it was far more limited. In these temperate regions there where clear genetic differentiation between sampled populations as well as a lack of isolation by distance.

In *B. dorsalis* Aketarawong *et al*. (2007) explored the genetic variation as well as the population structure in 14 populations distributed across the species range (Far East Asia, South Asia, Southeast Asia and the Pacific Area) using microsatellites. All populations showed high levels of genetic diversity and there was evidence for population differentiation. They showed structured groups for Bangladesh (East Asia), Myanmar (Southeast Asia) as well as Hawaii (Pacific). The other populations in Southeast Asia (excluding Myanmar) seem to be admixed and show high levels of gene flow within the region. Based on their samples they conclude that China is the possible source of *B. dorsalis*. *Bactrocera dorsalis* occurs sympatrically with *B. papayae* and *B. philippinenensis* within Asia (Thai/Malay region) (Schutze *et al*. 2012a; Krosch *et al*. 2013). Many studies have attempted to construct routes of invasion for *B. dorsalis* (Wan *et al*. 2011, 2012; Shi *et al*. 2012; Aketarawong *et al*. 2014). These studies showed that the tropical regions of Southeast Asia as well as the Southern coast of China is probably the native range of this species and that there are high levels of movement and interconnectivity among populations in East Asia. Khamis *et al*. (2009) used microsatellite markers to examine the genetic diversity and connection between African populations in *B. invadens* (now synonomous with *B. dorsalis*). They showed that African populations have high levels of gene flow, lack population differentiation and shows no evidence for bottlenecks. They conclude that there is a process of rapid expansion and multiple introductions at play.

Hu *et al*. (2008) made use of a mitochondrial marker (COI) to assess the phylogeography of *B. cucurbitae* in East Africa. They showed a homogenous distribution of genetic diversity across Southern China, Thailand, Philippines and Japan with one dominating haplotype (latter populations have been eradicated, Koyama *et al*. 2004). Using microsatellites Virgilio *et al*. (2010) investigated the macrogeographic population structure of *B. cucurbitae* from 25 sampling locations worldwide. They show that the species can be dividid into five groups:

samples from the African continent, La Réunion, Central Asia, East Asia and Hawaii. They also show the probability of inter-regional movement as well as a Central Asian origin for the species. Jacquard *et al*. (2013) further showed three distinct clusters in La Réunion, distinguishable from those in Africa and Asia with gene flow between the clusters.

In a Mediterranean-wide study in *B. oleae*, Augustinos *et al*. (2005) showed three subpopulations: the first the Iberian Peninsula, the second Greece and Italy and the third Cyprus, despite an indication of gene flow across this region. They also show possible expansion from the east to the west in this region based on the decrease of genetic variation to the west. Nardi *et al*. (2005) made use of mitochondrial and microsatellite markers to investigate the population structure and colonisation history of this species. They show that there is only a small amount of differentiation at regional scales in *B. oleae*, but clear differentiation on a continental scale. All evidence presented identified three groups: the first samples from Pakistan, the second samples from Africa and the third samples from the Mediterranean as well as America. They conclude that Africa is possibly the native range of this species rather than the Mediterranean. Moreover, Zygouridis *et al*. (2008) indicated that the eastern parts of the Mediterranean are probably the source population for populations in California. These Californian populations are also genetically distinct from the Mediterranean samples.

In *A. suspense*, Boykin *et al*. (2006) used COI to show that the species as a whole is monophyletic and that samples from Florida and the Caribbean region shows no population differentiation based on geographical location or host plants used. Results from microsatellites (Boykin *et al*. 2010) supported these findings of no differentiation between Florida and the Caribbean with continuous gene flow between them.

Integrated pest management of fruit flies in South Africa

Fruit flies have an increasing effect on fruit producers in South Africa, not only through direct damage to fruit (see Fig 1.2 for general life cycle) but also through quarantine restrictions on export limiting market access. South Africa's fruit export industry is highly profitable with an estimated annual earning of US$ 1 billion (Barnes 2000; Barnes & Venter 2006). The control of fruit flies in deciduous and citrus fruit is costly and the Western Cape Province (South Africa) spends roughly R 17 million on control each year.

Four different control techniques are implemented in fruit orchards in South Africa. The first is a bait application technique (BAT), which uses a combination of hydrolysed protein food bait and pesticide (for example: organophosphates, spinosad or synthetic pyrethroids) sprayed on a localized spot away from the fruit to attract and kill (Ekesi & Billah 2007). The second method is based on BAT, but the food bait and pesticide combination is placed in a container (trap) that localizes insecticidal exposure and retains the flies in the trap (Ekesi & Billah 2007). The third method involves full cover organophosphorous sprays at set time intervals, but because of the negative impacts of residues on human health and the environment, methods that are more environmentally-friendly are in high demand. The Sterile Insect Technique (SIT) is one such environmentally-friendly technique and is based on matings between mass-reared sterile males and wild females. The technique aims to decrease the size of the population to a threshold at which genetic forces that influence small populations, including genetic drift (Lande 1995; Lynch *et al*. 1995; Frankham 2005), and the loss of genetic diversity, drives the population to possible extinction (Bonizzoni *et al*. 2002).

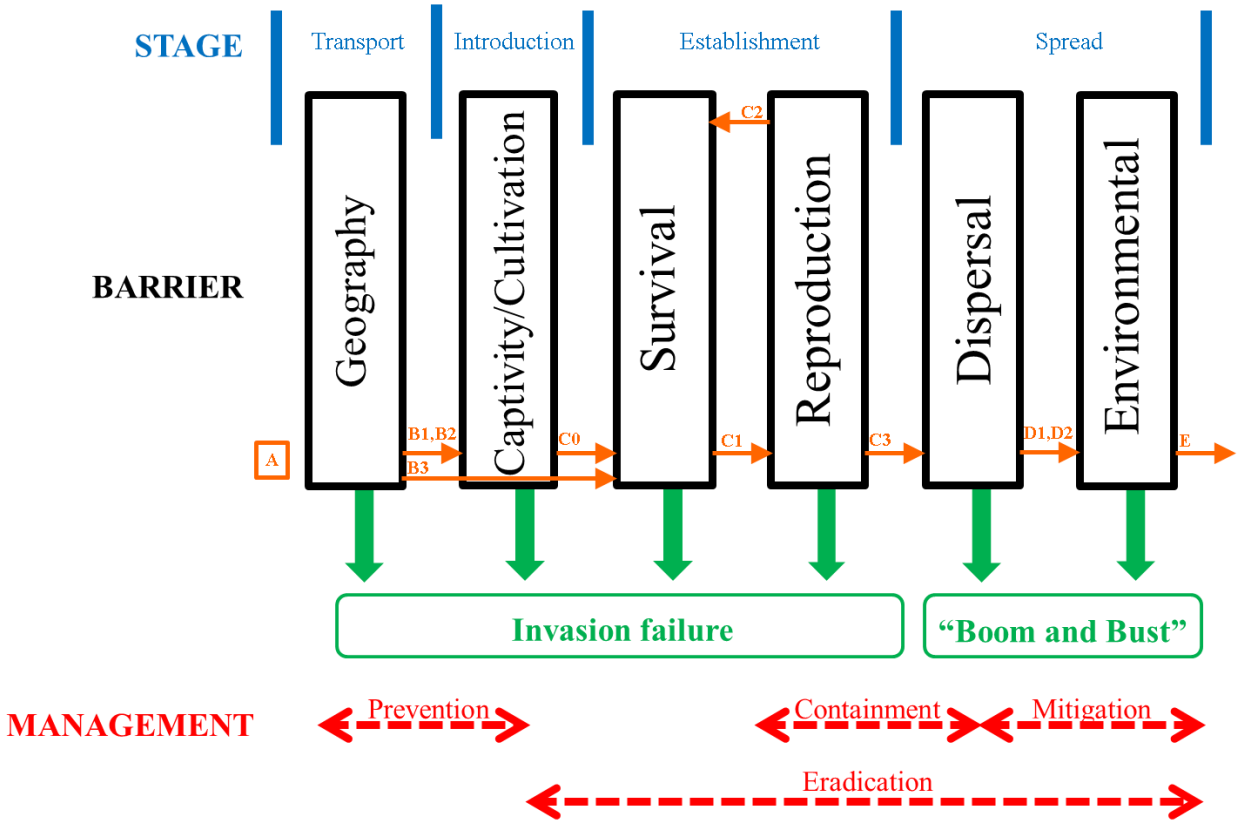


Figure 1.1 A simplified version of the proposed framework for biological invasions, redrawn from Blackburn *et al.* (2011). Their framework shows the different stages of the invasion process (in blue) and the barriers (in black) a species needs to overcome in each stage to be able to move on to the next stage, as well as the outcome (in green). There are also different management strategies (in red) for species in each of the different stages. Arrows and blocks in orange depict the movement of an organism through the framework with regards to the barriers A- not transported beyond native range; B1- individuals transported beyond native range limits in quarantine or captivity ; B2- individuals transported beyond native range limits in cultivation; B3- individuals transported beyond native range limits, released in novel environment; C0- individuals released into introduced location, cannot survive for long time, C1- individuals surviving in but not reproducing; C2- individuals surviving in wild, not self-sustaining population; C3- individuals surviving in wild, self-sustaining population; D1- self-sustaining population in wild, survives significant distance from the first point of introduction; D2- self-sustaining population in wild, survives and reproduces significant distance from the first point of introduction; E- species fully invasive, individuals surviving, dispersing and reproducing over multiple sites.

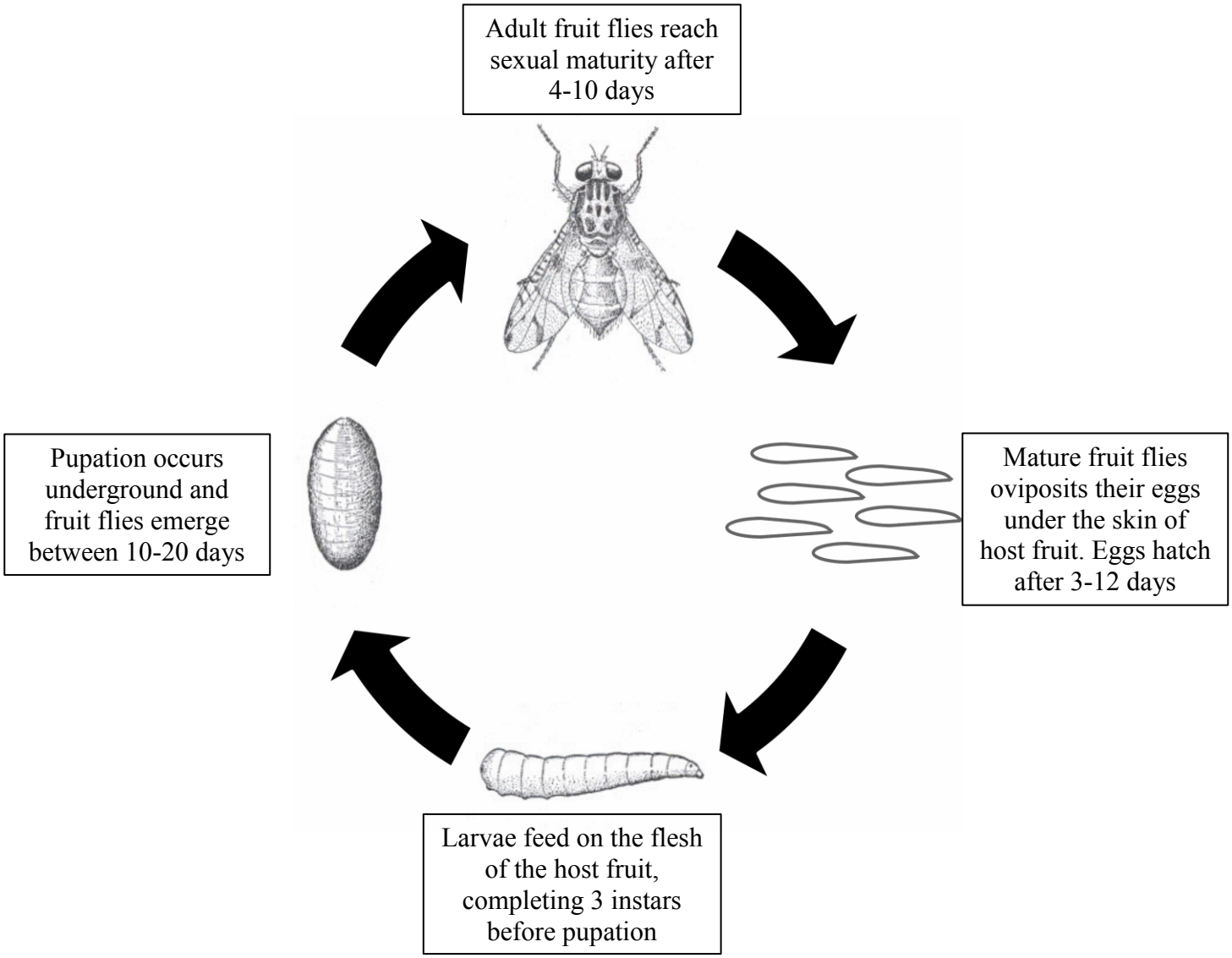


Figure 1.2 A schematic representation of the general life cycle of fruit flies (Diptera:Tephritidae) under laboratory conditions (25ºC). Drawings taken from Skaife (1953).

Some examples of successful SIT programs exist, for example tsetse flies (Diptera: *Glossina* spp.) and screw worms (Diptera: *Cochliomyia*), but in other taxa, for example in the codling moth, (Lepidoptera: Totricidae) SIT has shown only limited success (Lance & McInnis 2005). In South Africa an SIT pilot project for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) was started in 1999 in the Hex River Valley and was commercialized during 2004 (Barnes *et al*. 2002; Barnes & Venter 2006). No such project is currently underway for any other tephritid in South Africa including the Natal fruit fly, *Ceratitis rosa* (Karsch).

Three of the keystone components of an Integrated Pest Management (IPM) program, are 1) sampling, 2) pest identification and 3) understanding population dynamics of the pest (Pedigo 2002). Fundamental to any IPM program is to have a good monitoring system in place to facilitate early detection, to establish diversity of fruit flies in an area, and to estimate population abundance to assist decision-making. Monitoring all ports-of-entry and country boundaries are crucial for early detection and effective monitoring techniques are of vital importance especially in the first stages of biological invasions (see Fig. 1.1). One of the most important questions here are, therefore, how effective is our monitoring efforts and the tools used to monitor? South Africa largely makes use of traps baited with a lure (parapheromone) or food bait attractant to monitor population abundance. As different species of fruit fly can co-exist in the same orchard in combination with the high cost of monitoring, optimizing trapping, which can depend on a variety of factors especially lure efficacy over multiple species, are crucial.

In South Africa, two species from the genus *Ceratitis* MacLeay, *C. capitata* (Mediterranean fruit fly) and *C. rosa* (Natal fruit fly) are of economic importance. Unlike *C. capitata* with a near global distribution, *C. rosa* is largely restricted to eastern and southern Africa (Angola, Ethiopia, Kenya, Malawi, Mali, Mozambique, Nigeria, Rwanda, South Africa, Swaziland, Tanzania, Uganda, Zaire, Zambia, Zimbabwe) but has spread to the Indian Ocean Islands of Mauritius and La Réunion in the last 50 years (White & Elson-Harris 1994; De Meyer 2001). Both *C. capitata* (more than a 250 fruit hosts in Africa; Appendix I) and *C. rosa* (more than a 100 fruit hosts; Appendix II) has a polyphagous life-history (White & Elson-Harris 1994), damaging many crops of commercial value. The origin and native range of some fruit flies has been under debate with evidence for *C. capitata* pointing to an Afrotropical native range and support has been increasing that *C. rosa* shares this native range (Malacrida *et al*. 1998; De Meyer *et al*. 2002; Gasperi *et al*. 2002; Baliraine *et al*. 2004; De Meyer *et al*. 2008).

In a recent study, Karsten *et al*. (2013) showed that *C. capitata* in South Africa has high genetic diversity, but lacks population differentiation over local-, regional- and broad scales across the country. Results from this research raised some questions with regards to what gene flow looks like between South Africa and the rest of the world as well as whether South Africa in actual fact forms part of the native range of *C. capitata*. The population genetic structure of *C. capitata* has been investigated using a variety of genetic and biochemical markers (Gasperi *et al*. 2002; Bonizzoni *et al*. 2004; Alaoui *et al*. 2010). Studies performed across large geographical scales using genetic markers have shown that *C. capitata* populations globally can be subdivided into three groups. The first is the ancestral population from sub-Saharan Africa, the second is the population from the Mediterranean Basin and the third group are populations from Latin America and the Pacific (Gasperi *et al*. 2002). A pattern of isolation-by-distance is clear in some locations and within others there is still some genetic variation present from the ancestral populations, an indication of a recent range expansion or multiple introductions. Reconstructing and testing the invasion hypotheses of *C. capitata* is important as it can shed light on multiple factors that drive successful invasions but also help prevent them (Estoup & Guillemaud 2010).

The life history characteristics of *C. rosa* are very similar to that of *C. capitata* and concerns exist regarding the threat of *C. rosa* to fruit exports. Some of these characteristics include survival of *C. rosa* at high altitudes but also tolerance to colder environmental conditions than *C. capitata* (Duyck & Quilici 2002). In areas of overlapping distribution *C. rosa* has the potential to outcompete *C. capitata* (Duyck & Quilici 2002). Regardless of the economic importance and the potential threat to fruit export the population genetics, specifically the population genetic structure of *C. rosa* is not well understood. A few previous studies have investigated the population genetics and spread of *C. rosa*. Baliraine *et al*. (2004) showed that the natural populations in eastern and southern Africa (mainland) and recently introduced populations in Mauritius and La Réunion. They also showed that there is a lack of population differentiation on the African mainland, with some degree of differentiation in the island populations. They also indicate that these high levels of genetic variation on the mainland are indicative of large population sizes. This study, however, focused on the African continent with only a few samples from South Africa (samples from Burgers Hall, Somerset West and Nelspruit). Virgilio *et al*. (2013) showed two clear genetic clusters (R1 and R2 morphotypes) within *C. rosa* which, upon closer inspection, corresponded to morphological differences, especially clear in the feathering of the midtibia of the males (Fig. 1.3).

Figure 1.3 Midtibia of *Ceratitis capitata* males showing clear differences between the area covered by the dark colouration as well as the length of the feathering.

In addition to using genetic markers to investigate population structure, other markers such as morphological, behavioural and physiological characters have been shown to be important when assessing the different evolutionary forces that shape the phylogeographic structure of a species (Magniez-Jannin *et al*. 2000; Drotz 2003; Garnier *et al*. 2004). In some instances it has also been shown that where neutral molecular markers failed to identify differentiation between populations, morphology could reveal clear population structure (Nice & Shapiro 1999; Bouyer *et al*. 2007). Traditional morphometrics use methods based on size variation with more modern methods being based on shape (geometric morphometry) variation (Strauss & Bookstein 1982; Rohlf & Marcus 1993; Dryden & Mardia 1998). These traditional methods were based on the measurement of length, width and depth but does not contain a lot of information on shape and are rather ambiguous (Zelditch *et al*. 2004). Geometric morphometrics allows for analyses independent of size, position and orientation and has been applied to a wide range of fields, including morphology and fingerprint recognition, and is considered to be more heritable than size alone (Bitner-Mathé & Klaczko 1999; Dujardin *et al*. 2003). Moreover, wing shape has been shown to evolve rapidly (Weber 1990; Santos *et al*. 2004) and in *Drosophila* it is controlled by various additive genes (Weber *et al*. 1999; Zimmerman *et al*. 2000).

1.1 The aims and objectives of this dissertation

The overarching aim of this dissertation is to attain knowledge on basic and applied biology of invasive fruit flies that may feed into management plans for South Africa to prevent new invasions. Furthermore, to aid the development of effective integrated area-wide pest management control strategies for fruit flies of economic importance in South Africa for sustainable agriculture.

The specific objectives of each chapter are as follows:

1. to investigate seasonal variation in fruit fly abundance in orchards and natural vegetation in the Western Cape to determine whether natural vegetation is used as possible refugia;
2. to investigate global population structure of *C. capitata* with a focus on southern Africa to reconstruct and test *C. capitata's* invasion pathway using a Bayesian framework;
3. to investigate the population genetic structure, using molecular and morphological markers, to estimate gene flow and dispersal ability of *C. rosa* in South Africa.

Each chapter is written as a separate research paper, therefore some repetition and overlap may occur.

CHAPTER 2: MONITORING SEASONAL POPULATION FLUCTUATIONS OF *CERATITIS ROSA* AND *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN THE WESTERN CAPE, SOUTH AFRICA IN ORCHARDS AND NATURAL VEGETATION

2.1 Introduction

Agricultural pests have significant impacts on agricultural productivity which goes hand in hand with social, ecological and economic costs (Kirk *et al*. 2013). With an increase in human population the pressure on food security will rise even more (e.g. Gregory *et al*. 2005; Godfray *et al*. 2010; Bebber *et al*. 2013). Monitoring of agricultural pests can be used to determine geographical distribution, assess effectiveness of control measures as well as develop forecast methods (Dent 2000). Central to montitoring programmes are the sampling techniques used to measure changes in insect abundance which provides essential measures by which control decisions are made. Two of the most important challenges when monitoring agricultural pests are to monitor species over long enough time-scales and large enough areas as well as changes in the detectability of species over temporal and spatial scales. Effective monitoring techniques and information on the demography of agricultural pests is of vital importance for a successful integrated pest management (IPM) program as it provides an estimate of relative population abundance, which in turn is used to determine the level of control employed in an orchard or agricultural environment.

The Tephritidae constitute approximately 500 genera of 'true' fruit flies of which about 35% are fleshy fruit feeders, with the rest associated with flowers, leaves, stems or roots (White & Elson-Harris 1994). Two species in the genus *Ceratitis*, the Mediterranean fruit fly (*C. capitata* (Wiedemann)) and the Natal fruit fly (*C. rosa* Karsch) are common and widespread in South Africa and of great economic importance. Both species are polyphagous, attacking a wide variety of commercially grown fruit and are especially damaging on deciduous fruit (Annecke & Moran 1982; White & Elson-Harris 1994). However, many more species of fruit fly are known to occur in the region (White & Elson-Harris 1994), more specifically, members of the genus *Dacus* (for example *Dacus ciliatus* Loew) and *Bactrocera oleae* (Rossi) which are found in crops interspersed with deciduous fruit orchards. South Africa has a profitable deciduous fruit industry with approximately 76 000 ha under production, focused mainly in the Western Cape, with annual export earnings of *c*. $450 million (Barnes *et al*. 2002; Barnes & Venter 2006). Consequently, the control of fruit flies associated with direct damage as well as preventing these flies from making their way into export consignments is a high priority in ensuring international market access.

Currently South Africa implements various techniques for the control of fruit flies. These include the widely used bait application technique (BAT), bait stations, full-cover sprays

(Ekesi & Billah 2007) and, for *C. capitata*, restricted use of the Sterile Insect Technique (SIT) over parts of the Western Cape (Barnes & Eyles 2000). Assessing the use of various monitoring systems in an area is therefore critical especially since successful trapping is dependent on several factors, including climate, trapping method (e.g. trap type) and lure or attractant (parapheromone or food bait). In South Africa, more than one species of fruit fly from more than one genus can co-occur in the same orchard and are often monitored using the same lure. Moreover, the effective sampling range of different lures (from how far lures are able to attract flies) are also important and may vary depending on the host plant in which traps are hung (Epsky *et al*. 2010) as well as other abiotic factors. Many studies have investigated the attractiveness of various protein baits for different species, specifically for *C. capitata* (Epsky *et al*. 1995; Vargas *et al*. 2002; Fabre *et al*. 2003; Epsky *et al*. 2010; Peñarrubia-María *et al*. 2014). Very few however, have investigated the attractiveness of particular baits for coexisting *Ceratitis* spp..Thus, an additional challenge in monitoring is to understand lure efficacy across multiple species and across different climatic zones.

Different commercial protein baits have been evaluated for *C. capitata*, *C. rosa* and *C. cosyra* in mango (Grout *et al*. 2011) and citrus orchards (Manrakhan & Kotze 2011) in the subtropical Mpumalanga Province in South Africa. From these studies, Questlure and the 3- or 2-component Biolure seemed most attractive to *C. capitata* and *C. rosa* under the conditions tested (Grout *et al*. 2011; Manrakhan & Kotze 2011). Vargas *et al*. (2000) showed that varying weather conditions impact lure formulations causing some lures to lose their attractiveness prematurely or to be less volatile. No lure comparisons have yet been conducted in the Western Cape Province, South Africa, with its Meditterranean climate, although it is clear that lure responses differ under different climatic conditions (Suckling *et al*. 2008).

Multiple generations of *Ceratitis* spp. are observed in a year with flies typically completing their life-cycle within three to four weeks under ideal laboratory conditions (26 ºC) (Grout & Stoltz 2007). In South Africa different host plants are available year round and the main factor influencing fruit fly abundance seems to be related to temperature fluctuations, especially low temperatures (Nyamukondiwa *et al*. 2013). During winter, population numbers are suppressed with an increase in temperatures during spring and summer marking an increase in fruit fly numbers. This period of suppression may become shorter in future and may lead to longer periods of activity in fruit fly populations as global increases in

temperature, with higher frequencies of extreme temperature events, are experienced (Archer & Rahmstorf 2010; Hansen *et al*. 2012).

In South Africa *C. capitata* and *C. rosa* overwinter as adults in home gardens (De Villiers *et al*. 2013a), and has been argued to be plausible from a physiological perspective (Nyamukondiwa *et al*. 2013). Aluja *et al*. (1996) showed that *Anastrepha* populations in Mexico fluctuate between different orchards and that there seems to be continuous movement from neighbouring native vegetation with the most flies caught in traps on the periphery of a mango orchard. Given that both *C. capitata* and *C. rosa* infest a number of wild hosts not commercially grown and with fruit set during times when orchards are bare, they can serve as viable alternatives.

Therefore, the aims of this study were broadly two-fold. First, I sought to assess seasonal variation in Tephritid abundance in the Western Cape and to investigate the role of different lures to attract a range of potential fruit fly pests by making use of four different types of commercially-available lures. Second, by monitoring in both orchards and adjacent natural vegetation I aimed to test if natural vegetation might serve as areas of refugia for *C. capitata* and *C. rosa* and if small populations of fruit flies might survive the winter in these natural areas adjacent to orchards to provide insights into seasonal population fluctuations in their abundance. The expectation here is that if flies are abundant in natural vegetation at times of no host availability in orchards, then these may be acting as sources of re-invasion during the next fruit season. The alternative hypothesis is that when fly abundance is low in both habitat types at the same time point, then populations are genuinely suppressed.

2.2 Materials and Methods

2.2.1 Study sites and trapping

Trapping of fruit flies using baited traps (details below) was conducted between February 2012 and March 2013 in orchards and adjacent natural vegetation in the Western Cape Province, South Africa. A total of four orchards were selected: one each in the Witzenberg Valley (S33°16'37.48"; E19°14'2.09"), Villiersdorp (S34°0'9.07"; E19°17'40'), Brandwag (Hexriver Valley) (S33°37'4.78"; E19°24'12.10" and Franschhoek (S33°55'26.08"; E19°8'14.88") and three sites with natural vegetation, associated with the above orchards, in Franschhoek (S33°55'14.11"; E19°8'30.38"), Witzenberg (S33°16'7.34"; E19°13'42.32") and

Stellenbosch (S33°51'3.97"; E18°52'34.30"). For Witzenberg Valley and Franschhoek where I had sites in both habitat types the distance between plots in orchards and natural vegetation was respectively 1.06km and 0.55km.The vegetation in my natural sites largely consisted of Fynbos elements including Proteaceae, Ericaceae, Restionaceae, Asteraceae and Rustaceae as well as some invasive species including *Pinus* spp. at the Stellenbosch site there were also some members of the *Olea* family. A bucket trap (Chempac, Paarl Pty. Ltd, South Africa) baited with one of four different types of lure, were placed in orchards and adjacent natural vegetation with 50m between traps and from orchard or vegetation edges in each plot to prevent bait interference. Lures were chosen from two main groups of attractants, male lures and food baits, to attract fruit flies from multiple genera. To attract members of the genus *Dacus* I used Cue Lure (Chempac, Paarl, South Africa) which is a parapheromone that attracts males only, but with the added advantage that this lure attracts flies over long distances. Questlure (Eric Chem, Nexus, South Africa), Chempac Olive fruit fly lure (1,7-dioxaspiro[5,5]-undecane and ammonium bicarbonate), and the three-component lure, Biolure®, comprising of putrecine, ammonium acetate and trimethylamine (Chempac, Paarl, South Africa) are all food baits and attract both males and females. To specifically target *Bactrocera oleae*, but also assess its attractiveness for other members of the *Bactrocera* genus, Olive fruit fly lure was chosen. Questlure and Biolure were included to attract *C. capitata* and *C. rosa*, although Biolure® also attracts fruit flies more generally. A 1 x 1 cm strip of dichlorvos (Chempac, Paarl, South Africa) was placed in each trap to kill adult flies. Traps were serviced once every two weeks in the growing season and once a month thereafter. After collection, flies were identified, sorted and counted. The trap catch data by date (each time traps were serviced) were grouped into the four seasons, spring (September-November), summer (December-February), autumn (March-May) and winter (June-August). The autumn of 2013 only contains one sampling date in March after which sampling at all sites was terminated.

2.2.2 Data analyses

Although I set out to monitor fruit fly abundance more generally including species in the genus *Ceratitis*, *Bactrocera* and *Dacus* catches of members of the latter two genera were limited. A total of three *Dacus ciliatus* Loew, six *Bactrocera oleae* (Rossi) and six *Bactrocera biguttula* (Bezzi) were caught, largely in the natural vegetation site in Stellenbosch. Therefore, to assess the effects of different lures, different seasons and different

sites I compared the number of *C. capitata* and *C. rosa* flies only (too few other species were caught to analyse) for each of the sites. A subset of the original dataset was used to investigate the effect of different habitats (orchard vs natural) on relative abundance where I had sampled both orchards and natural vegetation at the same location (Franschhoek and Witzenberg Valley). To ascertain which of these study factors are most important to explain the trap catch data for each species I made use of generalized linear mixed models using penalized quasi-likelihood (PQL) for repeated sampling using the MASS package (Venables & Ripley 2002) in R (R Core Team 2013). The location variable was used as a random variable and all others (lure, season and site) were used as fixed effects. Summary graphs of trap catch data for different habitat types, lures and species were drawn using STATISTICA v12 (Statsoft Inc., Tulsa, Oklahoma).

2.3 Results

A total of 5728 *C. capitata* and 148 *C. rosa* individuals were collected over the sampling period (February 2012-March 2013) across all locations. Significantly more flies were caught during autumn 2012 (March-May) than in any of the other seasons (Table 2.1(a), (b); Fig. 2.1 (A), (B); Fig. 2.2 (A), (B)) including autumn 2013. Note however that since sampling ceased in March of 2013, only one month's worth of data was included in the autumn 2013 trap catch data and I therefore cannot exclude the possibility that if April and May 2013 sampling was included the numbers might be similar to autumn 2012.

For *C. capitata* there was a peak in trap catches between March and April with a decline in trap catch numbers towards winter (Fig. 2.2 (A)). *Ceratitis capitata* individuals were only captured again in January when trap catches steadily increased towards the autumn peak (Fig. 2.1 (A)). As with *C. capitata* there was a marked peak in trap catches of *C. rosa* in March with a decline in individuals caught towards winter (Fig. 2.1 (B)). However, *C. rosa* were detected much earlier (in spring) with a much less well-defined increase in trap catch numbers towards the autumn peak (Fig. 2.1 (B)).

Biolure® performed better overall throughout the different seasons for both *C. capitata* and *C. rosa* in orchards (Table 2.1(a), (b); Fig. 2.3 (A), (B)). Interestingly, Olive fruit fly lure also captured *C. rosa* individuals during Spring. In natural vegetation Biolure® captured more *C. capitata* and *C. rosa* individuals on average than any of the other lures (Fig. 2.4 (A), (B)). In autumn Questlure captured the highest numbers of *C. capitata* in natural vegetation followed

by Biolure® and Olive fruit fly lure (Fig. 2.4 (A)). Although I did not explicitly test for movement between orchards and adjacent natural vegetation I could compare fruit fly abundance between the two habitat types in Witzenberg Valley and Franschhoek. For both *C. capitata* and *C. rosa* abundance, I caught more individuals of both species in orchards than in natural vegetation in all seasons (Table 2.2; Fig. 2.2 (A), (B)). In paired orchard and natural vegetation sites the distance between the orchard and the natural vegetation did not influence the number of flies caught (Fig. 2.5 (A), (B); Fig. 2.6 (A) and (B)).

Table 2.1 Regression results of (a) *Ceratitis capitata* and (b) *Ceratitis rosa* trap catches using generalized linear mixed models with a penalized quasi-likelihood for a Poisson distribution.

(a)

Element	Slope ± SE	d.f.	*t*-value	*p*-value
Intercept	3.88±0.97	126	3.994	0.097
Lure: Cue Lure	-6.60±1.39	126	-4.738	0.000
Lure: Olive Fruit Fly	-5.14±0.67	126	-7.628	0.000
Lure: Questlure	-2.22±0.16	126	-13.522	0.000
Season: Winter 2012	-3.62±0.34	126	-10.577	0.000
Season: Spring 2012	-6.79±1.65	126	-4.120	0.000
Season: Summer 2012	-1.98±0.16	126	-12.471	0.000
Season: Autumn 2013	-2.06±0.16	126	-12.524	0.000

Lure: Biolure® *versus* Cue lure *versus* Olive fruit fly lure *versus* Questlure; Season: Autumn 2012 *versus* Winter 2012 *versus* Spring 2012 *versus* Summer 2012 *versus* Autumn 2013. Standardized within-group residuals: Q1 = -0.142, Median = -0.027, Q3 = -0.003. Random effects: Location, Standard deviation = 2.378.

(b)

Element	Slope ± SE	d.f.	t-value	p-value
Intercept	1.04±0.81	126	1.281	0.696
Lure: Cue Lure	-26.74±50071.36	126	-0.001	1.000
Lure: Olive Fruit Fly	-2.68±0.51	126	-5.276	0.000
Lure: Questlure	-1.58±0.31	126	-5.063	0.000
Season: Winter 2012	-4.58±1.39	126	-3.287	0.001
Season: Spring 2012	-2.02±0.41	126	-4.931	0.000
Season: Summer 2012	-1.18±0.29	126	-4.087	0.000
Season: Autumn 2013	-2.79±0.58	126	-4.785	0.000

Lure: Biolure® *versus* Cue lure *versus* Olive fruit fly lure *versus* Questlure; Season: Autumn 2012 *versus* Winter 2012 *versus* Spring 2012 *versus* Summer 2012 *versus* Autumn 2013. Standardized within-group residuals: Q1 = -2.330e-01, Median = -7.456e-02, Q3 = -9.710e-14. Random effects: Location, Standard deviation = 1.884.

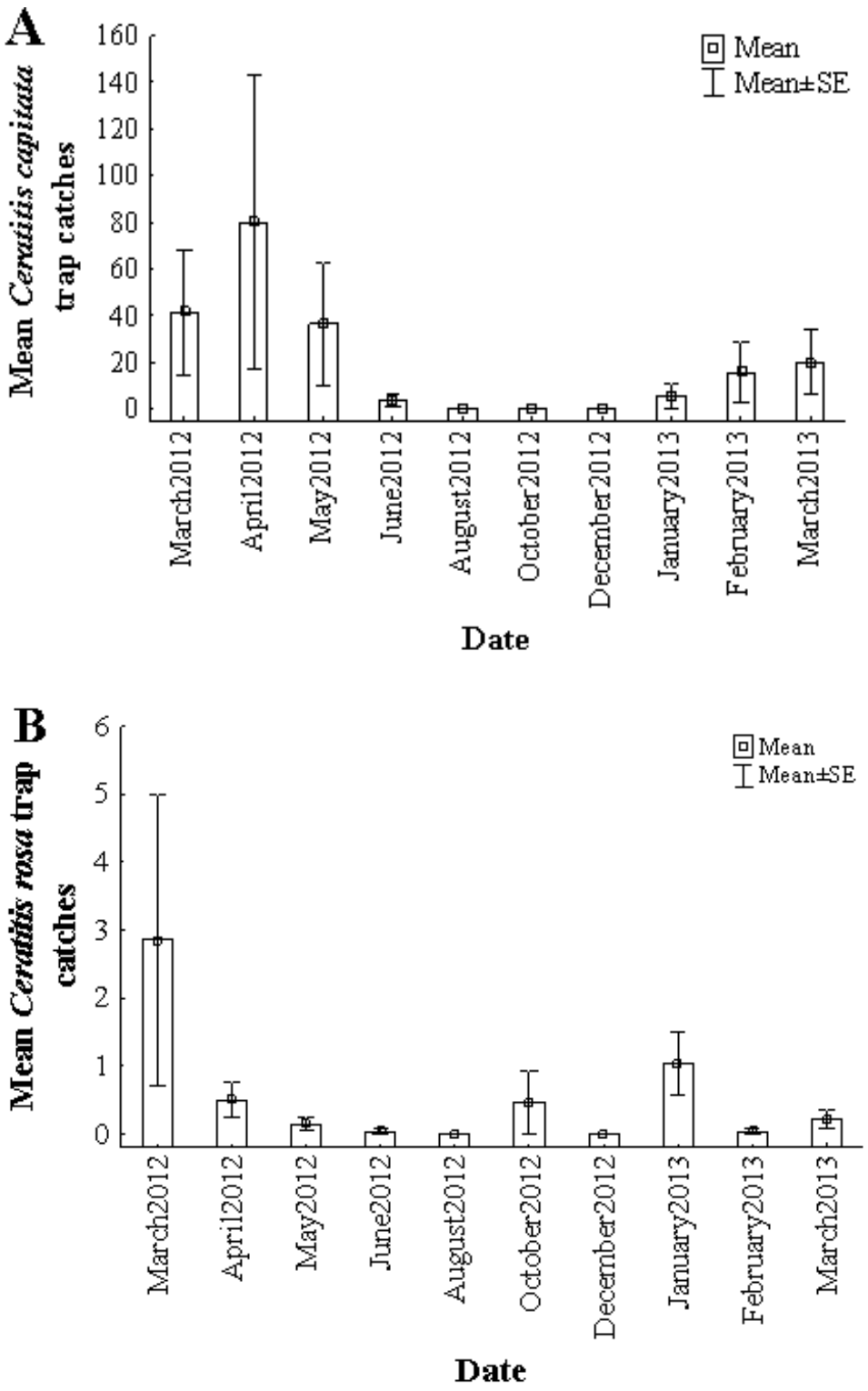


Figure 2.1 Temporal patterns of mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over all orchards and lures for each month that traps were serviced.

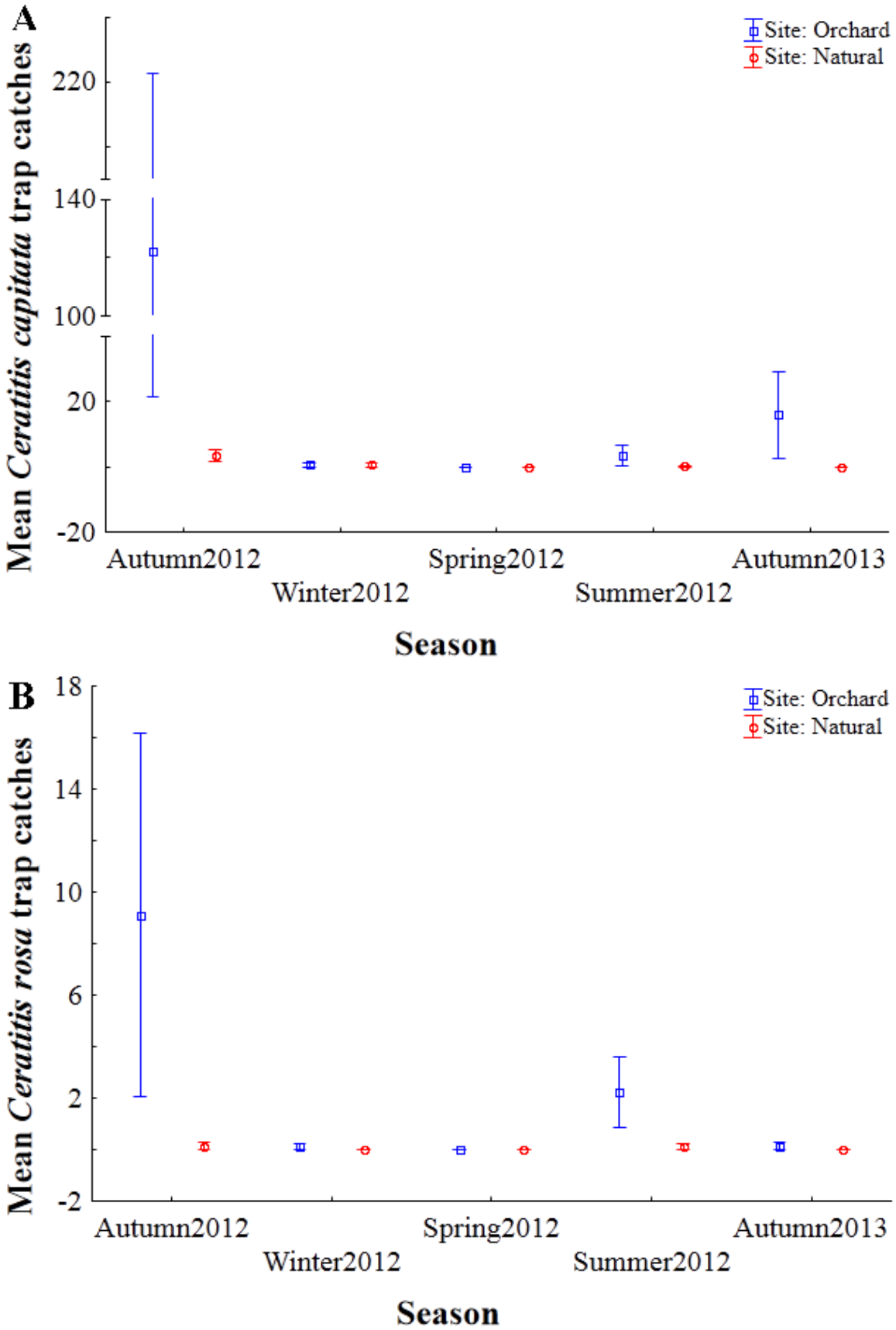


Figure 2.2 Mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over the different locations and lures for the two habitat types (orchard and natural) for all the seasons monitored.

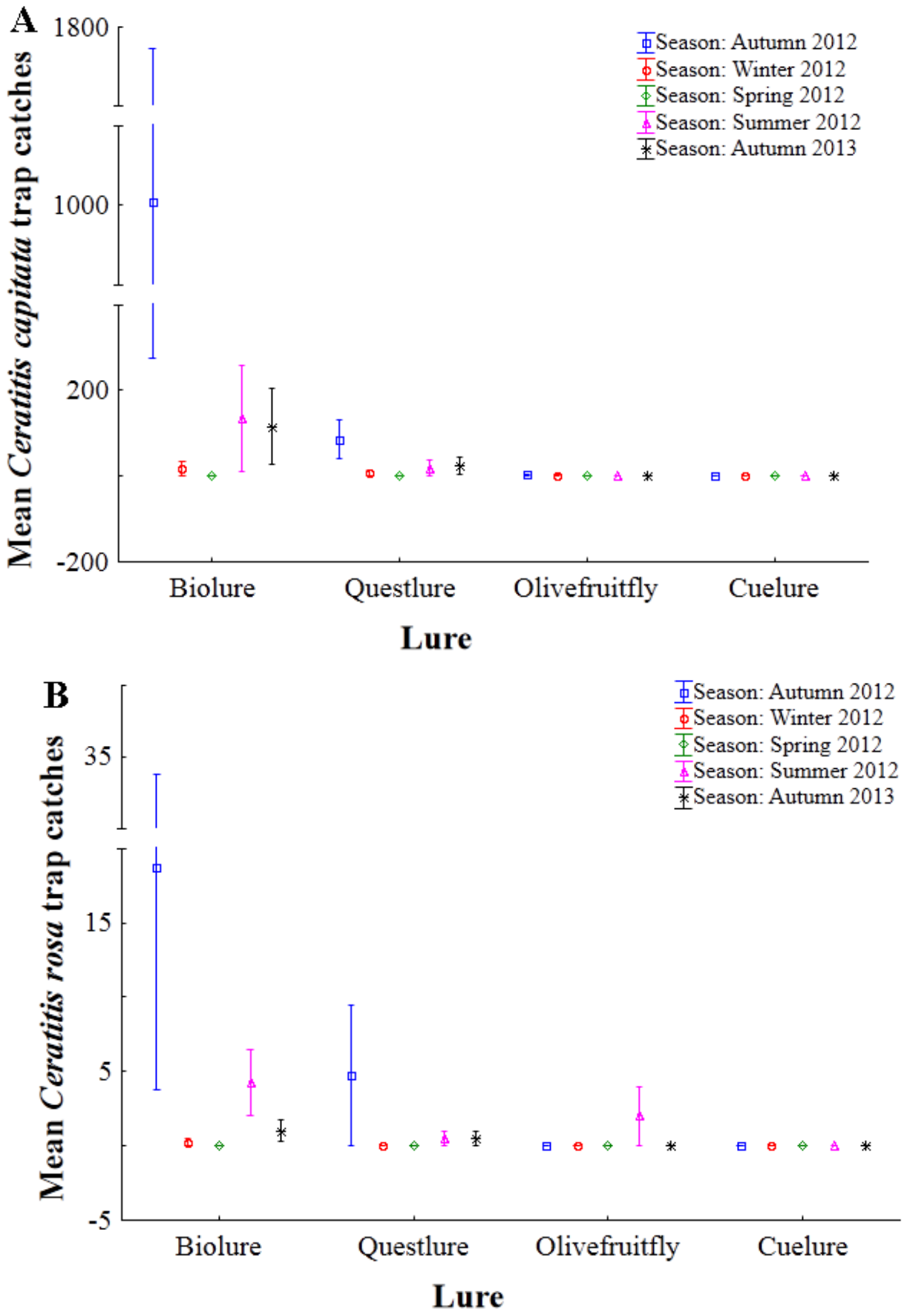


Figure 2.3 Mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over the four orchards (Witzenberg valley, Villiersdorp, Brandwag, Franschhoek) for the four lures (Biolure, Questlure, Olive fruit fly lure, Cue lure) for all the seasons monitored.

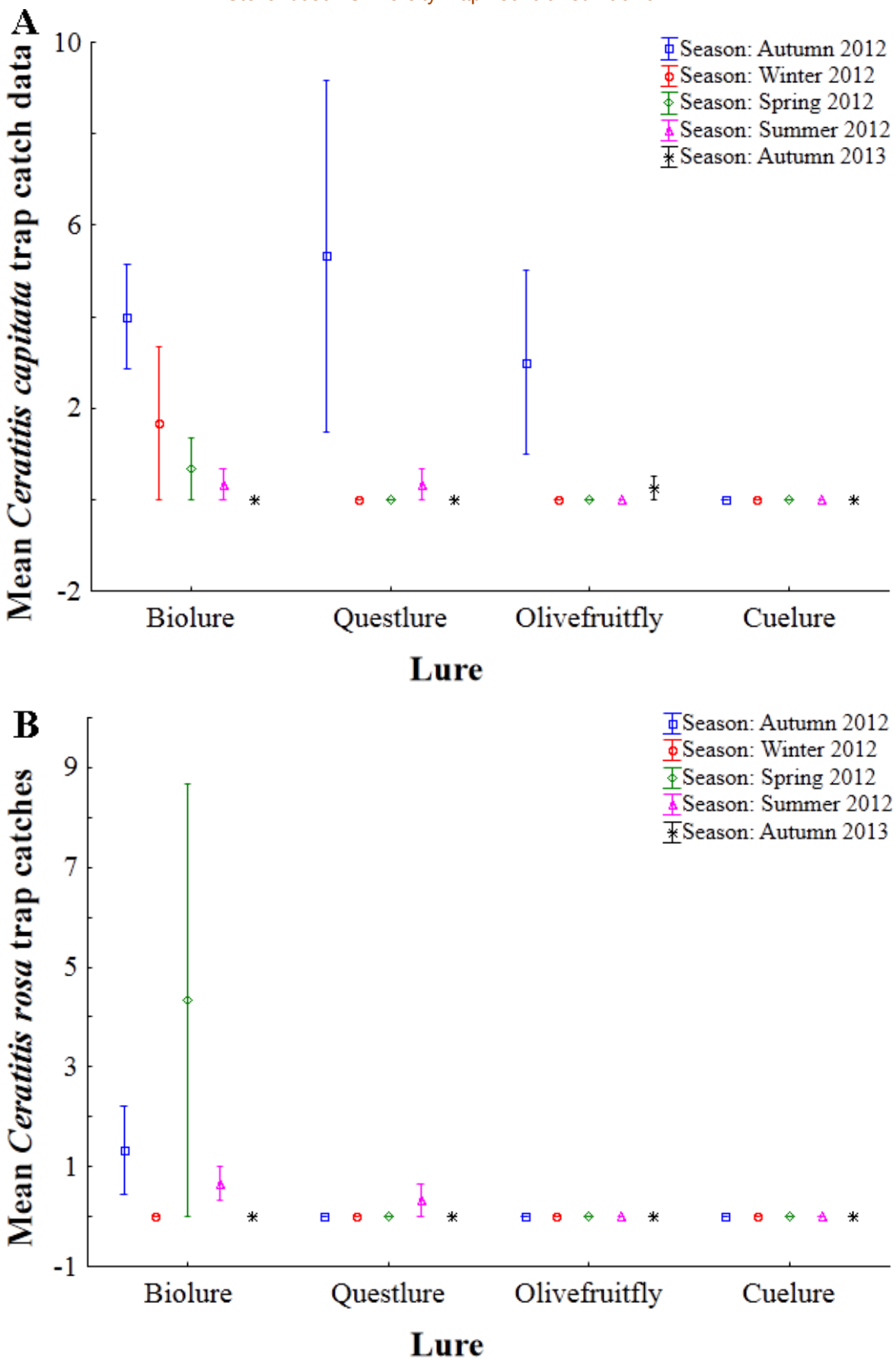


Figure 2.4 Mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over the three areas of natural vegetation (Witzenberg valley, Stellenbosch, Franschhoek) for the four lures (Biolure, Questlure, Olive fruit fly lure, Cue lure) for all the seasons monitored.

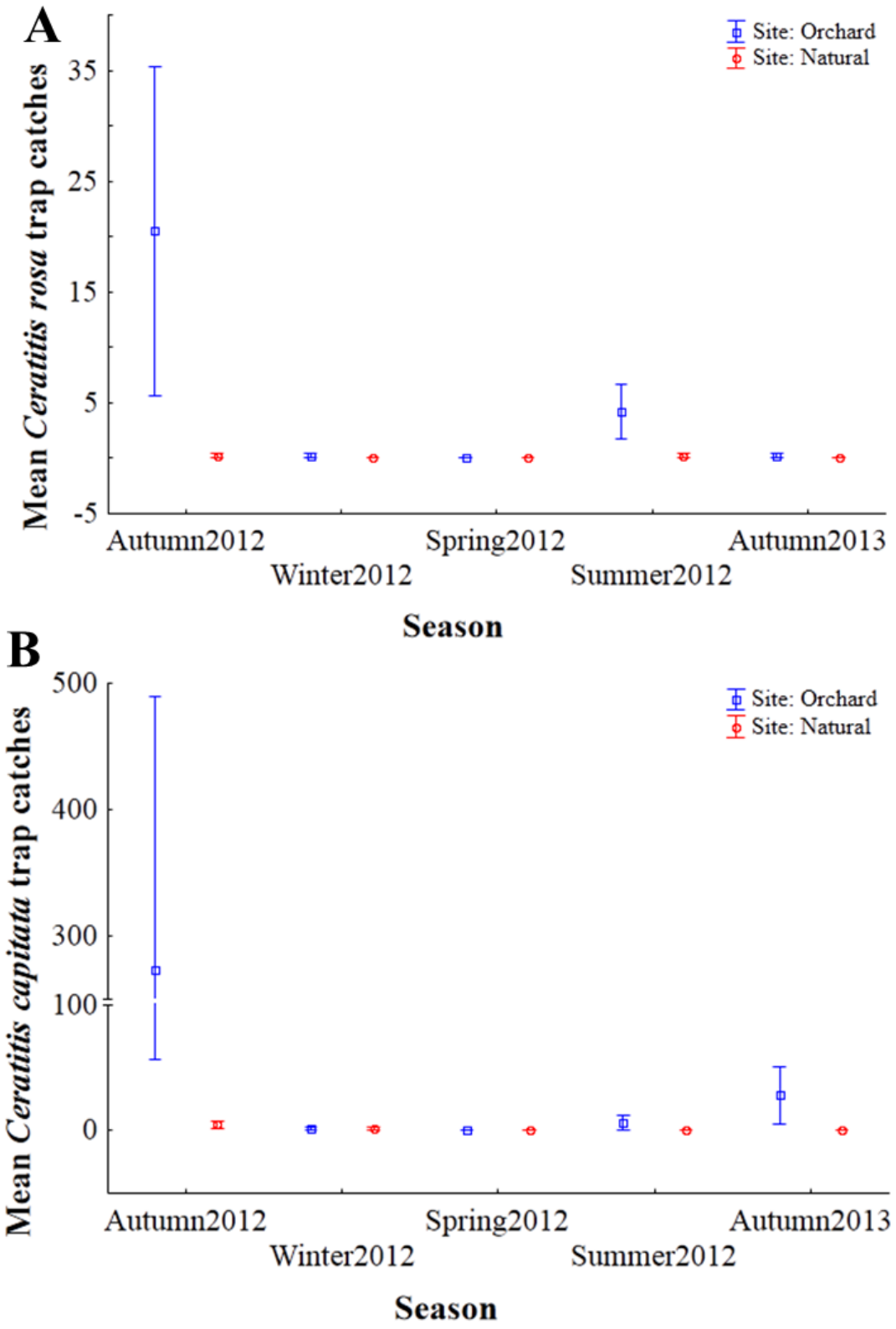


Figure 2.5 Mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over all lures for Franschhoek over the two habitat types (orchard and natural) for all the seasons monitored.

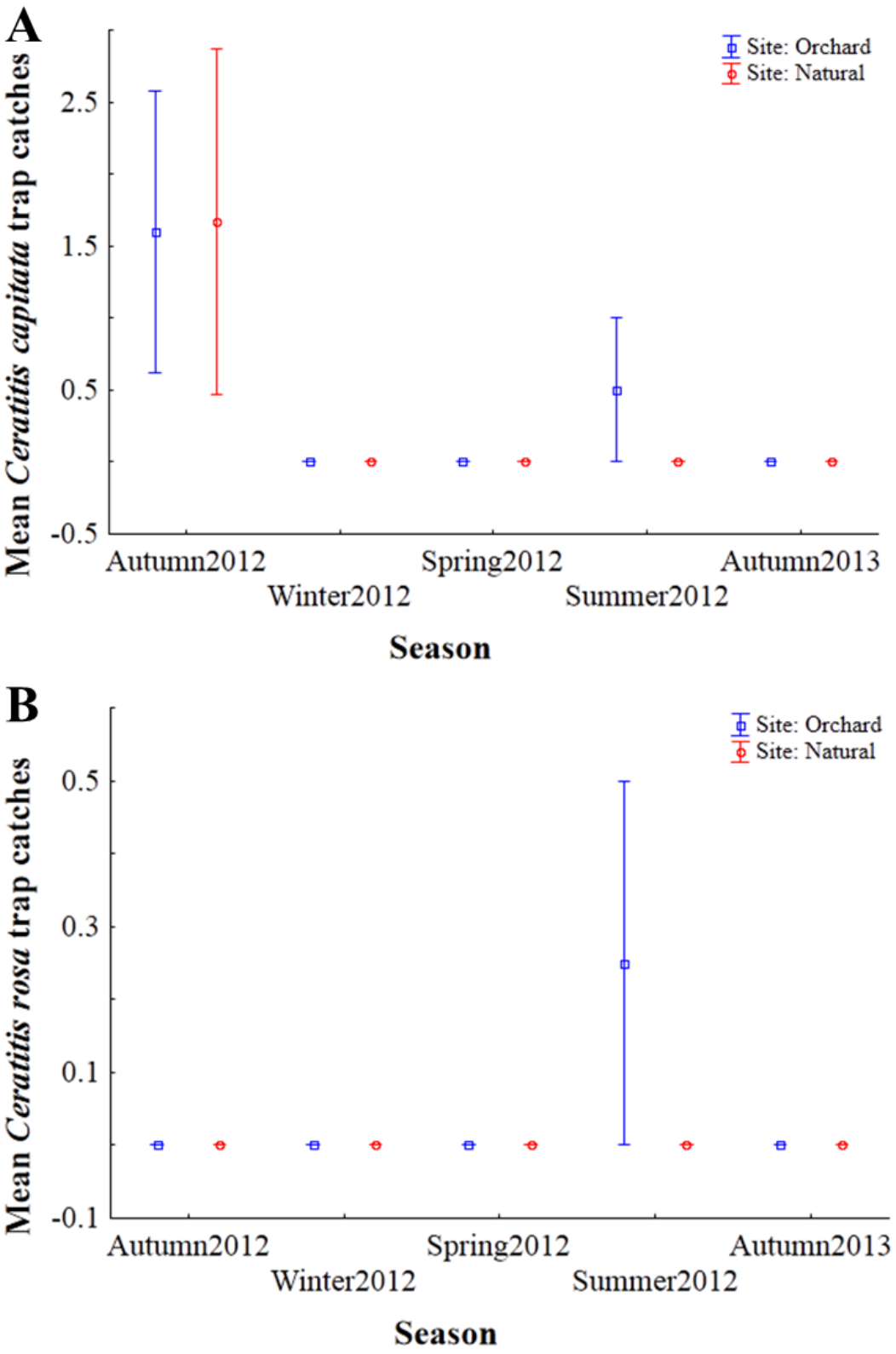


Figure 2.6 Mean (±SE) *Ceratitis capitata* (A) and *Ceratitis rosa* (B) trap catches averaged over all lures for Witzenberg valley over the two habitat types (orchard and natural) for all the seasons monitored.

Table 2.2 Regression results of trap catches of (a) *Ceratitis capitata* and (b) *Ceratitis rosa* between Witzenberg valley and Franschhoek using generalized linear mixed models with a penalized quasi-likelihood for a Poisson distribution.

(a)

Element	Slope ± SE	d.f.	*t*-value	*p*-value
Intercept	0.96±1.71	70	0.561	0.577
Lure: Cue	-5.86±1.4	70	-4.200	0.000
Lure: Olive Fruit Fly	-5.03±0.92	70	-5.489	0.000
Lure: Quest	-1.61±0.18	70	-8.835	0.000
Season: Winter 2012	-4.72±0.77	70	-6.160	0.000
Season: Spring 2012	-29.76±126368.5	70	0.000	1.000
Season: Summer 2012	-3.69±0.46	70	-7.997	0.000
Season: Autumn 2013	-2.31±0.24	70	-9.646	0.000
Site: Orchard	3.69±0.44	70	8.412	0.000

(b)

Element	Slope ± SE	d.f.	*t*-value	*p*-value
Intercept	-2.06±1.85	70	-1.114	0.269
Lure: Cue	-28.74±104709.4	70	0.000	1.000
Lure: Olive Fruit Fly	-2.27±0.32	70	-7.046	0.000
Lure: Quest	-1.4±0.22	70	-6.291	0.000
Season: Winter 2012	-4.42±0.87	70	-5.058	0.000
Season: Spring 2012	-1.47±0.22	70	-6.673	0.000
Season: Summer 2012	-30.13±201927.7	70	0.000	1.000
Season: Autumn 2013	-4.42±0.87	70	-5.057	0.000
Site: Orchard	3.93±0.62	70	6.340	0.000

Lure: Biolure® *versus* Cue lure *versus* Olive fruit fly lure *versus* Questlure; Season: Autumn 2012 *versus* Winter 2012 *versus* Spring 2012 *versus* Summer 2012 *versus* Autumn 2013; Site: Natural vegetation *versus* Orchard.

2.4 Discussion

In this study I found that Biolure® was the most effective lure overall for *C. capitata* and *C. rosa* with the highest trap catches for both species in autumn. I also showed that there was a significant difference between the two habitat types monitored (natural vs. orchard) with orchards typically containing much greater numbers of both species. Given the fact that a diverse array of lure types were used here, and considering the most effective lure type, both species were not trapped, and thus probably not abundant, in the natural vegetation adjacent to orchards. Care should however be taken as conclusions made here are based on limited seasonal sampling and I discuss enahancement of methods used here to reach more comprehensive conclusions.

The effectiveness of protein baits have been assessed in citrus orchards (Manrakhan & Kotze 2008) as well as in mango orchards (Grout *et al*. 2011) in Mpumalanga Province, South Africa, for *Ceratitis* spp. and showed that Biolure® was highly effective in attracting *C. capitata*, *C. rosa* and *C. cosyra*. The same was also true in La Réunion where Peñarrubia-María *et al*. (2014) showed that Biolure® was highly effective in capturing both *C. rosa* and *C. capitata*. My findings therefore support this growing body of support for the use of Biolure®. Efficacy of lures used in monitoring systems is critical for estimates of relative population abundance, as this is not only used to monitor fruit fly numbers in orchards for determining economic thresholds for population control (e.g. Broughton & De Lima 2002), but also to screen for possible new invasions (e.g. Simberloff 2009b). This assumption has, however, been called into question as an established population can exist at undetectable levels using traditional monitoring methods (Papadopoulos *et al*. 2013). The relative attractiveness of a particular lure to a specific species is therefore critical in implementing successful monitoring.

Similarly to Manrakhan & Addison (2014) I had the highest trap catches in autumn which corresponds to the fruit phenology of deciduous fruit commercially grown in the Western Cape. During the rest of the year trap catches dwindled close to zero, although some overwintering individuals seemed to be common in the region (see also Nyamukondiwa *et al*. 2013). From trap catch results shown here it seems reasonable to conclude that flies are likely not moving from orchards into natural vegetation as trap catches were the highest in autumn in both habitat types, and instead that there is probably a process of local population growth

and decline, or perhaps also re-colonisation of orchards from home gardens, annually. To more accurately assess the seasonal fluctuations in relative abundance of *C.capitata* and *C. rosa* in this area future studies should include several replicates of the temporal variable (season) and sample multiple years. The low numbers of *C. rosa* and *C. capitata* individuals caught in natural vegetation is arguably due to the lack of fleshy fruits and berries in the Fynbos elements that dominated my natural vegetation habitat type. To assess whether these flies are able to sustain populations in natural vegetation possible natural hosts need to be collected and checked for fruit fly emergence. Although it is clear that home gardens are used as refugia when preferred host plants are not available (De Villiers *et al*. 2013a) there are also other biotic and abiotic factors that influence the relative abundance of both species in the region. An important abiotic factor for example is climate, including seasonal temperatures, amount of annual rainfall and to some extent, probably also relative humidity. Therefore, measuring some of these abiotic factors in addition to monitoring fruit fly numbers would be valuable to assess the impact of these factors on relative abundance of these species as well as a possible effect on lures. Another important factor in relative abundance of fruit flies is management practices in orchards *versus* the lack of control of fruit flies in other habitats which can serve as the origin of new infestations (Segura *et al*. 2006). In this region it seems that different or patchy management practices in combination with varying levels of orchard sanitation may play a much larger role in relative abundance of the two species than natural vegetation harbouring large populations during winter.

As most commercial orchards are well managed with rigid spray programs the number of fruit flies caught may differ depending on how many applications they have. Spray programs are often abandoned after harvest of deciduous fruit and this in combination with poor sanitation, most likely corresponds to the peak in fruit fly numbers that I see in my data during autumn. For a successful IPM program care should therefore be taken to monitor fruit fly abundances even after the season has ended as this peak in numbers serves as the pool from which orchards will be reinvaded in the next season. Finally, it would appear that Biolure® may accurately reflect the population composition of the two fruit fly species, when compared to previous extensive fruit sampling data.

CHAPTER 3: DECONSTRUCTING INTERCONTINENTAL INVASION PATHWAY HYPOTHESES OF THE MEDITERRANEAN FRUIT FLY (*CERATITIS CAPITATA*) USING A BAYESIAN INFERENCE APPROACH: ARE PORT INTERCEPTIONS AND QUARANTINE PROTOCOLS SUCCESSFULLY PREVENTING NEW INVASIONS?

3.1 Introduction

With the increase in human population numbers placing rising pressure on food security together with the predicted effects of climate change on the productivity of agricultural areas (e.g. Gregory *et al*. 2005; Godfray *et al*. 2010; Bebber *et al*. 2013), research on the different biotic and abiotic factors influencing the likelihood, frequency and potential impacts of invasions are increasing (Blackburn *et al*. 2011; Kirk *et al*. 2013). Arthropods (including the insects) are generally understudied in invasion ecology, but especially so on the African continent (Pyšek *et al*. 2008). Invertebrate pests move around the world via different human-mediated pathways (reviewed in Hulme 2009) and after establishment expand their range through natural dispersal in the newly colonized region. These human-mediated pathways are closely linked to trade of commodities and include transport by air and sea as well as subsequent movement on land by, for example, rail, road and canals (Hulme 2009; see also Gaston *et al*. 2003; Frenot *et al*. 2005).

After the initial introduction to the new location, the species avoiding or overcoming the geographic barrier, faces many additional challenges to be able to successfully establish (Blackburn *et al*. 2011). It is generally accepted that propagule pressure (number and frequency of individuals introduced at a specific location over time (Lockwood *et al*. 2009)), is the most important driver of successful invasions and not necessarily species-level characteristics (Lockwood *et al*. 2005; Lockwood *et al*. 2009; Simberloff 2009a; Blackburn *et al*. 2013). Szűcs *et al*. 2014 showed that although propagule pressure is important in the initial establishment, genetic processes are important for subsequent spread and growth of the population. Predicting what species will become invasive has been an urgent and longstanding priority in invasion biology (e.g. Kimberling 2004; Lloret *et al*. 2005; Van Kleunen *et al*. 2010; Philibert *et al*. 2011; Higgins & Richardson 2014) together with improved predictive models based on adequate biological data for well-developed control methods (Kirk *et al*. 2013). The number of possible biological input parameters is extensive, but of importance are traits characterising environmental niches, such as physiological parameters (e.g. thermal development requirements or activity limits) and dispersal capacity and/or distance travelled by natural or assisted means (Berthouly-Salazar *et al*. 2013). Furthermore, traits of population demographics, which may be linked to environmental niches (e.g. Dixon *et al*. 2009; Irlich *et al*. 2009), are also significant, since rapid population growth rates can readily assist in establishment and evolutionary adaptation post introduction (Gilchrist *et al*. 2008; Foucaud *et al*. 2010; Rey *et al*. 2012).

Some of the most successful invaders worldwide are part of the Tephritidae (for example: *Ceratitis capititata*, Malacrida *et al*. 2007; *Bactrocera dorsalis*, Aketarawong *et al*. 2007; *Bactrocera invadens*, De Meyer *et al*. 2010) constituting a group of agricultural pest insects, commonly referred to as the "true fruit flies", comprising more than 5000 species (White & Elson-Harris 1994). These fruit flies cause high levels of economic losses due to both direct damage of feeding larvae in fruit, as well as indirectly by placing restrictions on the export of fruit to certain trade partner countries. Therefore, the range expansion by natural or human-assisted forces are receiving substantial research attention in determining the native range and movement patterns of species specifically for the development and implementation of risk assessments as well as successful control programs (Leung *et al*. 2002; Stohlgren & Schnase 2006).

Possibly the most notorious and well known of the Tephriditae flies is *Ceratitis capitata* (Weidemann), the Mediterranean fruit fly, a highly successful invader worldwide possibly due to its large larval host range, broad tolerance to climatic conditions and its high dispersal capacity (Malacrida *et al*. 1992; Lance & McInnis 2005; Nyamukondiwa & Terblanche 2009; Nyamukondiwa *et al*. 2010). *Ceratitis capitata* is of quarantine importance worldwide (Reyes & Ochando 2004; De Meyer *et al*. 2008) and has spread to many countries outside of its native range, which is believed to be Afrotropical (De Meyer *et al*. 2002). Although Kenya (East Africa) has been identified as the likely native range (Malacrida *et al*. 2007) there still remains some uncertainty as to the extent of the native range within Africa as levels of genetic diversity for South Africa (Karsten *et al*. 2013) are similar to those estimated for Kenya and does not follow the decline in genetic variability seen in other derived populations worldwide (e.g. Bonizzoni *et al*. 2004; Malacrida *et al*. 2007). This raises some important questions, (1) is this high level of genetic diversity, lack of structure and high numbers of private alleles due to South Africa being part of the historical native range of *C. capitata* and (2) if South Africa is part of the native range, where does South Africa fit into the global population structure of *C. capitata*?

Population genetics can be used to investigate the colonization process, routes of invasion and the underlying evolutionary forces that shape the population structure of tephritids (e.g. Bonizzoni *et al*. 2001, 2004; Baliraine *et al*. 2004; Nardi *et al*. 2005; Gilchrist & Meats 2009; Aketarawong *et al*. 2014). Studies performed across large geographical scales using genetic markers have shown that *C. capitata* populations globally can be subdivided into three groups. The first is the ancestral population from sub-Saharan Africa, the second is the

population from the Mediterranean Basin and the third group are populations from Latin America and the Pacific (Gasperi *et al*. 2002). The colonization history of *C. capitata* worldwide is relatively well documented (Gasperi *et al*. 2002; Malacrida *et al*. 2007). The proposed invasion pathway and historical chronological order of colonization events includes firstly an introduction of *C. capitata* to the coast of the Mediterranean area, possibly human-mediated (Maddison & Bartlett 1989), consequently moving to Spain and the rest of the Mediterranean region (Fimiani 1989). This was followed by a secondary introduction to Australia from Europe in the 1890's (Hooper & Drew 1989; Bonizzoni *et al*. 2004). The next leg of introductions in *C. capitata's* global colonization was into the Americas, (Costa Rica) spreading south to Guatemala and along the coffee belt to Mexico (Harris 1989). Given the nature of these records and observations these dates all likely reflect the earliest record of *C. capitata* in the location, but not necessarily the date of introduction. This incongruity can be due to fruit flies establishing in an area long before they are detected in routine monitoring. In the past, support for proposed routes of invasion have come from genetic diversity estimations, where the area with the highest level of genetic diversity would be assumed the native range (Malacrida *et al*. 2007) or from gene flow estimates based on Slatkin's private allele method (Malacrida *et al*. 1998). However, these prior estimates are potentially weakened by the reduction of genetic data to a single summary statistic of diversity, which does not necessarily account for stochastic population events (Estoup & Guillemaud 2010). The importance of understanding invasion pathways, possible barriers and the ability to reconstruct pathways is made clear in the multiple practical implications especially surrounding the control of new invasions (i.e. risk management), as well as their prevention (Wilson *et al*. 2009; Estoup & Guillemaud 2010; Blackburn *et al*. 2011). For example, reconstruction of invasion pathways can assist in uncovering the native range which can, in turn, provide possibilities for biological control as natural enemies are typically sourced from the native range (Estoup & Guillemaud 2010). To date, however, hypotheses of macrogeographic colonization of *C. capitata*, have not been examined and tested using an *a priori* hypothesis-based, strong inference, Bayesian framework (e.g. Pascual *et al*. 2007).

Here I make use of microsatellites, genetic diversity estimates and concurrent geographic sampling at broad scales to investigate the population structure and routes of invasion of *C. capitata* with a strong focus on sub-Saharan Africa. I hypothesise that, based on previous studies of global *C. capitata* structure (Malacrida *et al*. 1998; Gasperi *et al*. 2002; Malacrida *et al*. 2007) there will be a clear decrease in genetic diversity moving away from East Africa towards the rest of the world, as well as genetic differentiation between native populations in

Africa and derived populations elsewhere in the world due to founder effects and genetic drift. Moreover, I investigate four distinct plausible hypotheses for the invasion pathway of *C. capitata* using approximate Bayesian computations (ABC). All hypotheses considered are based on an "out-of-Africa" approach including information from *C. capitata* macrogeographic studies and historical information of colonization. The first hypothesis I test is an introduction from Africa (all locations sampled here) to the Mediterranean region (Madeira and Greece), with a secondary introduction from the Mediterranean region to Australia and finally an introduction from Africa to Guatemala. Similarly I tested the same hypothesis but included an unsampled population on the Mediterranean coast from which from which the rest of the Mediterranean region was invaded. The next hypothesis was similar to the first, but the west African population (Senegal) is excluded and is tested as a separate introduction event. Finally I test whether South Africa falls outside of the native range of *C. capitata* and test separate introduction events to West Africa (Senegal) and South Africa.

3.2 Materials and Methods

3.2.1 Sampling and microsatellite genotyping

Ceratitis capitata individuals (*N*=323) were obtained from 14 populations worldwide with more extensive sampling focused on sub-Saharan Africa with between six and thirty individuals sampled per location (Table 3.1). Flies were collected via trapping in orchards using baits as well as reared from infested fruit. I refer to individuals from the same sampling location as a population in the rest of this study. After morphological confirmation of the identification of *C. capitata*, DNA was extracted using a DNeasy® tissue kit (Qiagen Inc.). All individuals collected were genotyped for 11 microsatellite markers following Karsten *et al*. (2013). In each plate I included a negative and a positive control to check that plates were read consistently between different runs. Samples were genotyped using an ABI 3130 Automated Sequencer (Applied Biosystems, Foster City, California, USA). Samples that remained unamplified after two independent reactions were assumed non-amplifiable and therefore not included. Four of the South African sites were part of the Karsten *et al*. (2013) study. Only a small subset was genotyped again and all individuals were scored again with the additional samples included.

3.2.2 Microsatellite analysis

3.2.2.1 Estimates of sample variability

I made use of GENEMAPPER v3.7 (Applied Biosystems, Foster City, California) to score alleles. All microsatellite markers were tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium (HWE) using 10 000 permutations in GENEPOP v4.01 (Raymond & Rousset 1995; Rousset 2008). In all cases where multiple testing were done I adjusted significance levels using False Discovery Rates (FDR; QVALUE, Storey 2002) and these are the values reported. Genetic diversity levels were investigated calculating basic statistics for all populations including: expected heterozygosity (H_E), observed heterozygosity (H_O), number of alleles (N_A), number of private alleles (N_P), inbreeding coefficient (F_{IS}), as well as allelic richness (A_R; genetic diversity independent of sample size) (GENETIX v4.05.2, Belkhir *et al*. 2004; GenAlEx v6.5, Peakall & Smouse 2006, 2012; FSTAT v2.9.3.2, Goudet 2002). The frequency of null alleles (A_n) was estimated in FREENA v1.0 (Chapuis & Estoup 2007). Furthermore, to assess whether the populations are expanding or contracting I made use of Bottleneck v1.2.0.2 (Cornuet & Luikart 1996) implementing both the two-phase model of mutation (TPM) and the stepwise mutation model (SMM). I ran 10 000 permutations and made use of one-sided Wilcoxon rank tests to determine whether populations showed deviations (excess or deficit) from expected heterozygosity.

Table 3.1 The locations of collection sites of *Ceratitis capitata* used in this study as well as sample size (N), number of alleles (N_A), number of private alleles (N_P), expected (H_E) and observed (H_O) heterozygosity (± standard error), allelic richness (A_R), the inbreeding coefficient (F_{IS}) and the mean null allele frequency (A_n, Dempster *et al*. 1977; SD in parentheses).

Country		ID	GPS coordinates		N	N_A	N_P	H_E	H_O	A_R	F_{IS}	A_n
			Latitude	Longitude								
Africa												
Kenya		KEN	-1.29	36.83	27	10.727	0.636	0.796±0.149	0.533±0.143	5.657	0.348	0.139 (0.087)
Mozambique		MOZ	-19.13	33.43	22	9.818	0.364	0.809±0.113	0.606±0.201	5.698	0.276	0.115 (0.110)
Senegal		SEN	14.18	-16.56	9	3.909	0	0.588±0.136	0.511±0.247	5.569	0.194	0.061 (0.107)
South Africa												
	Burgers Hall	BUR	-25.06	31.05	10	3.273	0.273	0.431±0.224	0.404±0.173	2.877	0.134	0.023 (0.053)
	Levubu	LV	-23.05	30.17	29	11.273	0.636	0.793±0.126	0.575±0.188	5.571	0.292	0.115 (0.103)
	Port Elizabeth	PE	-33.96	25.57	6	4.909	0.182	0.641±0.205	0.530±0.277	3.927	0.274	0.068 (0.090)
	Stellenbosch	CL	-33.88	18.74	30	11.273	1.182	0.814±0.080	0.637±0.188	5.787	0.235	0.099 (0.100)
	Upington	UP	-28.45	21.24	29	9.818	0.455	0.779±0.103	0.611±0.202	5.65	0.233	0.098 (0.083)
Tanzania		TAN	-6.95	37.53	28	11.273	0.727	0.819±0.091	0.662±0.214	5.138	0.211	0.095 (0.106)
Zimbabwe		ZIM	-17.86	31.04	23	10.273	0.273	0.800±0.113	0.603±0.212	5.228	0.269	0.116 (0.117)
Australia		AUS	-31.98	115.88	30	3.091	0	0.378±0.232	0.303±0.254	2.26	0.214	0.064 (0.095)
Europe												
Greece		GRE	39.4	21.91	28	2.727	0	0.369±0.228	0.287±0.245	2.147	0.244	0.070 (0.112)
Madeira		MAD	32.67	-16.84	22	3.364	0.091	0.487±0.176	0.319±0.197	2.718	0.373	0.125 (0.101)
Americas												
Guatemala		GUA	14.89	-90.51	30	2.909	0	0.391±0.193	0.359±0.218	2.217	0.102	0.047 (0.070)

3.2.2.2 Population structure analysis

I quantified the degree of population differentiation between populations by calculating pairwise F_{ST} values in MICROSATELLITE ANALYSER v4.05 (MSA; Dieringer & Schlötterer 2003) as well as overall F_{ST} values in FREENA v1.0 running 10 000 replications including (INA) and excluding (ENA) null alleles (Chapuis & Estoup 2007). The hypothesis of isolation by distance (IBD) for the African continent as well as the introduced range was investigated using two separate Mantel tests in ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010).To further assess the relationships between different populations based on their allele frequency I used a Principal Coordinate Analysis (PCoA) implemented in the program GenAlEx v6.5 (Peakall & Smouse 2006, 2012). The first three principal axes were plotted in STATISTICA v12 (Statsoft Inc., Tulsa, Oklahoma).

STRUCTURE v2.3.4 (Pritchard *et al*. 2000; Falush *et al*. 2003) was used to assign individuals to populations (genetic clusters) based on multilocus genotypes without including *a priori* information. The number of optimal clusters (*K*) was estimated using the admixture and no-admixture models with correlated allele frequencies which allow allele frequencies to be similar in different populations due to shared ancestry or continued migration. For each possible cluster (*K*), a number varying between 1 and 14 (i.e. the total number of sampling localities), I performed 10 independent runs with the burn-in set to 100 000 and 1 000 000 MCMC permutations. The optimal *K* (most likely number of clusters) was assessed using two methods. First, I inspected the log-probabilities of the different possible clusters; a high value with limited variance is an indication of the true *K* (Pritchard *et al*. 2000). Second, I inspected the second order rate change of Ln P(X/Y) calculated according to Evanno *et al*. (2005) implemented in the online resource STRUCTURE HARVESTER (Earl & Von Holdt 2012). As this method only detects the upper limit of structure when hierarchical structure exists, I also investigated substrucutring in my data by reanalysing main clusters. Due to possible IBD, I further assessed the assignment of individuals using TESS v2.3 (Chen *et al*. 2007; Durand *et al*. 2009) in which spatial coordinates of populations can be used as *a priori* information. The optimal *K* was determined running 10 independent runs for 200 000 MCMC iterations (burn-in= 20 000) using the admixture and no-admixture models and varying *K* between 2 and 14. I used the lowest deviance information criterion (DIC) value to choose the optimal *K*. Thereafter the runs (from STRUCTURE and TESS) of the chosen *K* value was averaged in CLUMPP v1.2.2 (Jakobsson & Rosenberg 2007) and visualized in DISTRUCT v1.1 (Rosenberg 2004).

I constructed unrooted neighbour-joining trees and assessed statistical support for each branch using non-parametric bootstrapping (10 000 replicates) using the online POPTREEW (Takezaki *et al*. 2014) based on Nei's genetic distance (D_A; Nei *et al*. 1983) as well as D_S, the genetic distance calculated based on the proportion of shared alleles (Bowcock *et al*.1994). To further investigate the genetic breaks in my sampling I made use of a hierarchical analysis of molecular variance (AMOVA) implemented in ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010) using 10 000 permutations. I partitioned the different sampled populations into five scenarios based on geographic subdivision, coancestry (results from STRUCTURE) and genetic relationships (from neighbour-joining trees).

3.2.2.3 Population demography analysis

Population demography was assessed in GENECLASS v2.0 (Piry *et al*. 2004) by making use of multilocus genotypes to calculate the probability of an individual being a resident of the population in which it was sampled versus being from any of the other reference populations. I made use of methods described in Rannala & Mountain (1997) as well as running the simulation algorithm according to Paetkau *et al*. (2004) for 10 000 genotypes and using a threshold of 0.01 as the probability of assignment. Furthermore, to obtain in depth information regarding the invasion pathway of *C. capitata* I tested four different hypothetical scenarios and analysed them using the ABC method implemented in the program DIYABC v2.03 (Cornuet *et al*. 2014). The use of ABC has many advantages including using all the data simultaneously as well as providing probability values with confidence intervals (Estoup & Guillemaud 2010). Scenarios were based on historical information as well as results from my cluster analyses. A detailed description of prior distributions of parameters used in the analyses can be found in Table 3.2. My timing of events was based on the number of generations *C. capitata* can support in a year and colonization dates available. I assumed that the effective population size was the same in all sampled populations and the bottleneck event (db) occurred after the initial introduction. The newly introduced individuals might take several generations to establish a population and the parameter was therefore bounded between one and ten generations. In total I considered twelve scenarios to test different hypotheses for the route of invasion, specifically that *C. capitata* originated in eastern and southern Africa and subsequently spread to the rest of the world. In each test I simulated 3 000 000 computations and for each scenario computed a posterior probability, including 95% confidence intervals (CI), with a logistic regression (Cornuet *et al*. 2014). The scenario with the highest posterior probability and non-overlapping 95% CI was chosen as the most

likely scenario. The chosen scenario was further evaluated by estimating Type I and II errors following Cornuet *et al*. (2010). The Type I error was calculated as the fraction of cases that the scenario tested (when it is true), does not have the highest probability. Conversely, the Type II error was calculated as the number of times that the tested scenario has the highest probability, when it is not the true scenario.

Table 3.2 Definitions and prior distributions of parameters used in the testing of three invasion route scenarios of *Ceratitis capitata* using the ABC method implemented in DIYABC v2.03 (Cornuet *et al*. 2014).

Parameter		Interval
Effective population size	Ni (i=1,2,…6)	Uniform [10-1 000 000]
Number of founder individuals in colonization event	Nib (i=1,2,…6)	Uniform [2-500]
Timing of events (generations back in time)		
	t1	Uniform [10-500]
	t2, t3	Uniform [100-1500]
	t4	Uniform [1000-10 000]
Duration of the bottleneck event (db)		[1-10]
Admixture rate (ra)		[0.001-0.999]
Mean mutation rate		Uniform [10^{-4}-10^{-3}]
Locus mutation rate		Gamma [10^{-5}-10^{-2}, 2]
Mean coefficient P		Uniform [0.1-0.3]
Locus coefficient P		Gamma [0.01-0.90, 2]
Mean SNI rate		Log-Uniform [10^{-8}-10^{-5}]
Locus SNI rate		Gamma [10^{-9}-10^{-4}, 2]

3.3 Results

3.3.1 Population genetic diversity

Overall my *C. capitata* populations showed deviations from HWE possibly due to the levels of inbreeding (F_{IS}) ranging between 0.102 (Guatemala) and 0.373 (Madeira) (Table 3.1) as well as the Wahlund effect (reduction in heterozygosity due to hierarchical structuring) and/or the presence of null alleles. The frequency of null alleles in all populations ranged between 0.023 and 0.139 (Table 3.1, Appendix III), but all loci were included in further analyses as there was no difference in F_{ST} estimation before and after null allele correction (see *Population structure* section). Furthermore, no linkage disequilibrium was detected for any of the 11 microsatellite markers. All African populations represented relatively high levels of genetic diversity based on the expected heterozygosity (H_E), number of alleles (N_A) and allelic richness (A_R) compared to those locations in the introduced range (Australia, Greece, Guatemala, Madeira). All African populations except Senegal had a number of private alleles (0.182-1.182; Table 3.1). Stellenbosch (South Africa) showed the highest number of private alleles with many locations (Australia, Greece, Guatemala, Senegal) having no private alleles. No recent bottlenecks (significant heterozygosity excess) were detected in any population based on results from the one-sided Wilcoxon ranks test (Table 3.3). Some populations in South Africa did, however, show significant heterozygosity deficit (Burgers Hall, Levubu, Stellenbosch, Upington), indicative of an expanding population.

3.3.2 Population structure

Genetic differentiation was measured by overall and pairwise F_{ST} calculations (Table 3.4). Pairwise F_{ST} values ranged between 0.014 (Stellenbosch and Upington) and 0.399 (Australia and Senegal) with only a small percentage (21%) of pairwise comparisons being non-significant, notably between Kenya and the remaining five African populations. The overall F_{ST} value before null allele correction (F_{ST}=0.143) as well as after ENA correction (F_{ST}=0.142) indicated significant population differentiation. There was no significant pattern of IBD based on results from the Mantel test for either the African continent (r=0.2706, p=0.175) or for the introduced range (r=-0.0578, p=0.640).

Table 3.3 The probabilities of a one-tailed Wilcoxon signed-rank test in 14 populations of *Ceratitis capitata* to validate significant heterozygosity excess (expanding) or deficit (contracting).

	TPM		SMM	
Location	deficit	excess	deficit	excess
Australia	0.382	0.650	0.120	0.897
Burgers Hall	0.188	0.839	**0.042**	0.984
Greece	0.615	0.423	0.313	0.722
Guatamala	0.813	0.216	0.216	0.813
Kenya	0.926	0.087	0.139	0.88
Levubu	0.260	0.768	**0.002**	0.998
Madeira	0.840	0.183	0.319	0.711
Mozambique	0.861	0.160	0.650	0.382
Port Elizabeth	0.449	0.584	0.319	0.711
Senegal	0.959	0.051	0.618	0.416
Stellenbosch	0.618	0.416	**0.002**	0.998
Tanzania	0.681	0.350	0.051	0.959
Upington	0.584	0.449	**0.008**	0.994
Zimbabwe	0.861	0.160	0.260	0.768

Table 3.4 Pairwise F_{ST} values calculated in MICROSATELLITE ANALYSER v4.05 (Dieringer & Schlötterer 2003) between 14 populations of *Ceratitis capitata*.

	AUS	BUR	GRE	GUA	KEN	LV	MAD	MOZ	PE	SEN	CL	TAN	UP	ZIM
Australia	0.000													
Burgers Hall	**0.282**	0.000												
Greece	**0.236**	**0.263**	0.000											
Guatemala	**0.277**	**0.266**	**0.097**	0.000										
Kenya	**0.231**	**0.158**	**0.220**	**0.221**	0.000									
Levubu	**0.237**	**0.129**	**0.234**	**0.229**	0.009	0.000								
Madeira	**0.245**	**0.241**	**0.235**	**0.243**	**0.162**	**0.164**	0.000							
Mozambique	**0.281**	**0.186**	**0.275**	**0.269**	0.019	0.022	**0.199**	0.000						
Port Elizabeth	**0.352**	**0.230**	**0.384**	**0.376**	0.061	0.065	**0.270**	0.069	0.000					
Senegal	**0.399**	**0.253**	**0.357**	**0.346**	**0.097**	**0.096**	**0.275**	**0.112**	0.104	0.000				
Stellenbosch	**0.276**	**0.187**	**0.268**	**0.270**	**0.030**	**0.028**	**0.201**	**0.030**	0.058	**0.108**	0.000			
Tanzania	**0.258**	**0.161**	**0.240**	**0.235**	0.017	0.014	**0.175**	0.016	0.058	**0.103**	**0.025**	0.000		
Upington	**0.279**	**0.178**	**0.282**	**0.284**	**0.033**	**0.036**	**0.202**	**0.037**	0.062	0.113	**0.014**	**0.031**	0.000	
Zimbabwe	**0.239**	**0.137**	**0.233**	**0.237**	0.011	0.007	**0.170**	0.017	0.048	0.105	0.022	0.012	**0.025**	0.000

Values in bold are significant ($p<0.05$)

In the Principal Coordinate Analysis (Fig. 3.1) the first 3 axes explained most of the genetic variation (63.22%). The first axis (45.33%) separates samples from Africa from the rest of the world including one sampling location from South Africa (Burgers Hall). The second axis separates the African group and the "world" (Australia, Greece, Guatemala, Madeira) group into two more clusters. Moreover, detailed population structure of the different populations can be investigated using the Bayesian clustering method implemented in STRUCTURE and TESS. Results from the admixture and no-admixture models were identical, here I therefore only present results from the admixture model. Graphical representation of the results from Evanno *et al*. (2005) (Fig. 3.2) indicated that K=2 was the optimal number of clusters in STRUCTURE (Fig. 3.3 (A); see Appendix IV). These results were further supported by identical clustering in TESS (Fig. 3.3 (B)) based on the lowest DIC value. The first cluster grouped all African populations together, except for Burgers Hall (South Africa), which groups more closely with populations from the introduced range. This peak at K=2 also corresponded to the results from the first axis of the PCoA (Fig. 3.1) separating African sampling localities (excluding Burgers Hall) with those from the rest of the world. This clustering corresponded to the unrooted neighbour-joining trees reconstructed based on D_S (proportion of shared alleles) and Nei's distance (Fig. 3.4) forming two clear groups. These two clusters were further investigated by running each cluster separately (Fig 3.5). Sub-structuring in the African cluster showed two further clusters based on the optimal K calculated in Evanno *et al*. (2005). Although clustering of the different populations to the two clusters was not clear. Within the world cluster three additional clusters were identified. The first cluster included Australia, the second individuals from both Greece and Guatemala, the third individuals from Madeira and the final cluster included all individuals from Burgers Hall. Moreover, I tested the homogeneity of populations using an AMOVA in which populations were clustered according to 5 different scenarios described in Table 3.5. There were significant differences among groups in all scenarios tested.

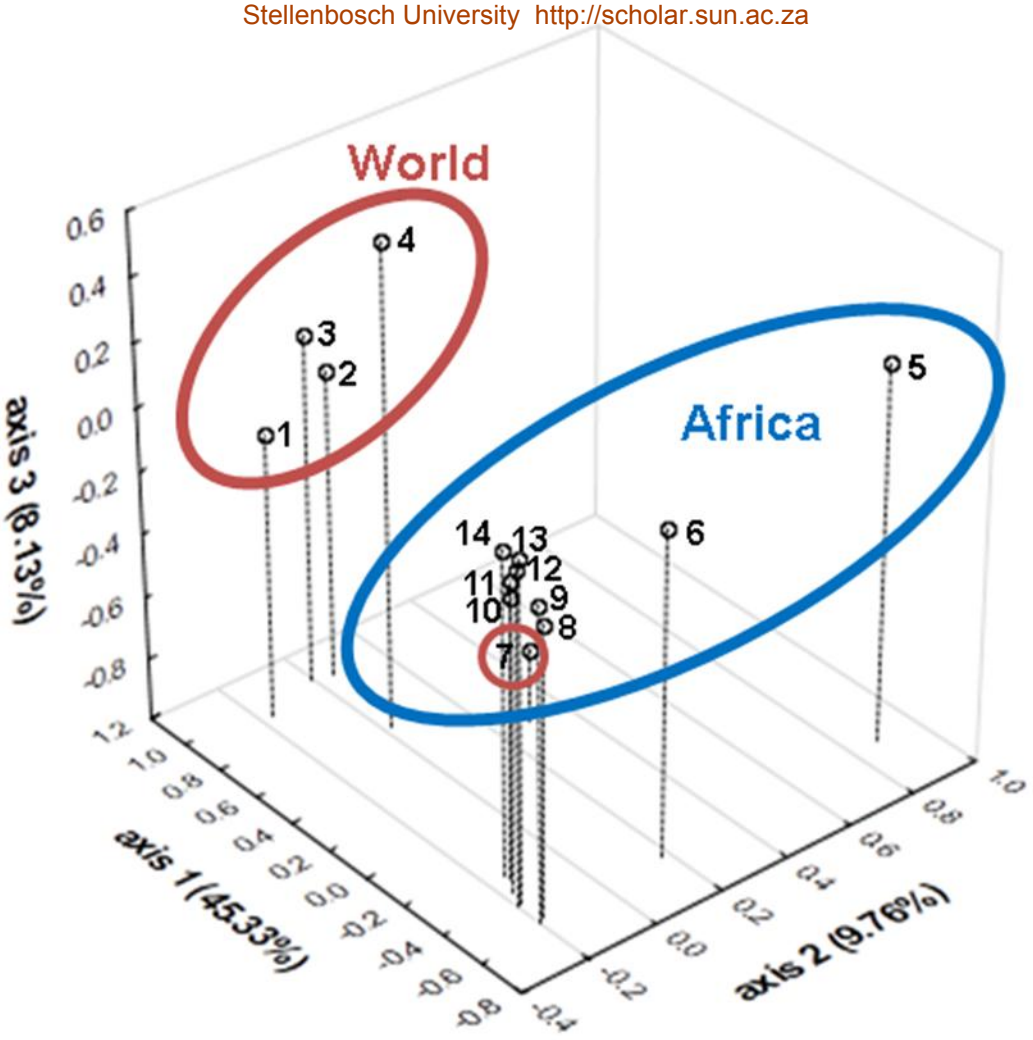


Figure 3.1 A three-dimensional plot of the Principal Coordinate Analysis (PCoA) for 14 *Ceratitis capitata* populations. 1- Australia, 2- Greece, 3- Guatemala, 4- Madeira, 5- Senegal, 6- Port Elizabeth, 7- Burgers Hall, 8- Upington, 9- Stellenbosch, 10- Zimbabwe, 11- Levubu, 12- Mozambique, 13- Tanzania, 14- Kenya. Coloured circles indicate the two clusters that correpond to clusters obtained in STRUCTURE, TESS and the NJ trees.

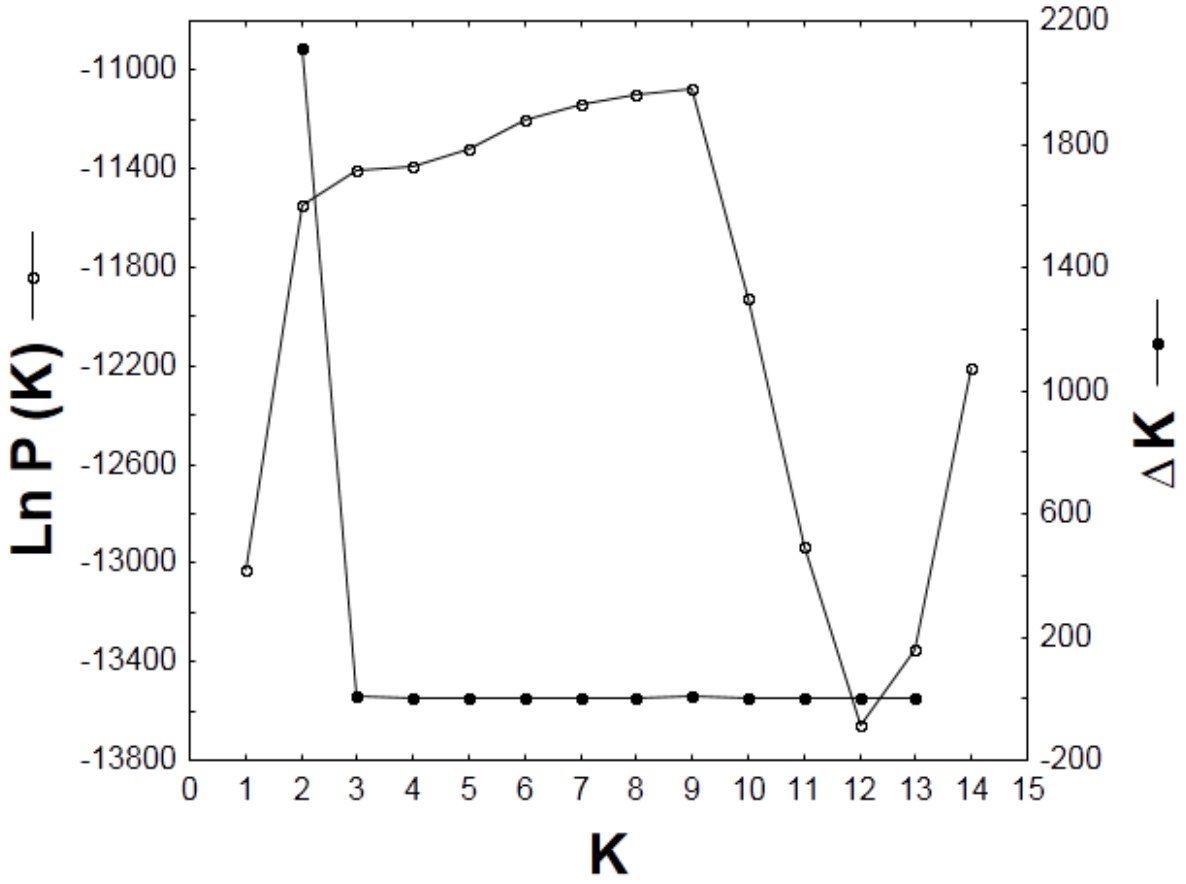


Figure 3.2 The lines indicate the most likely number of clusters (*K*) in STRUCTURE of *Ceratitis capitata* populations according to the natural logarithm of the likelihood (Ln P(X/K) (Pritchard *et al*. 2000) and the delta *K* method (Evanno *et al*. 2005).

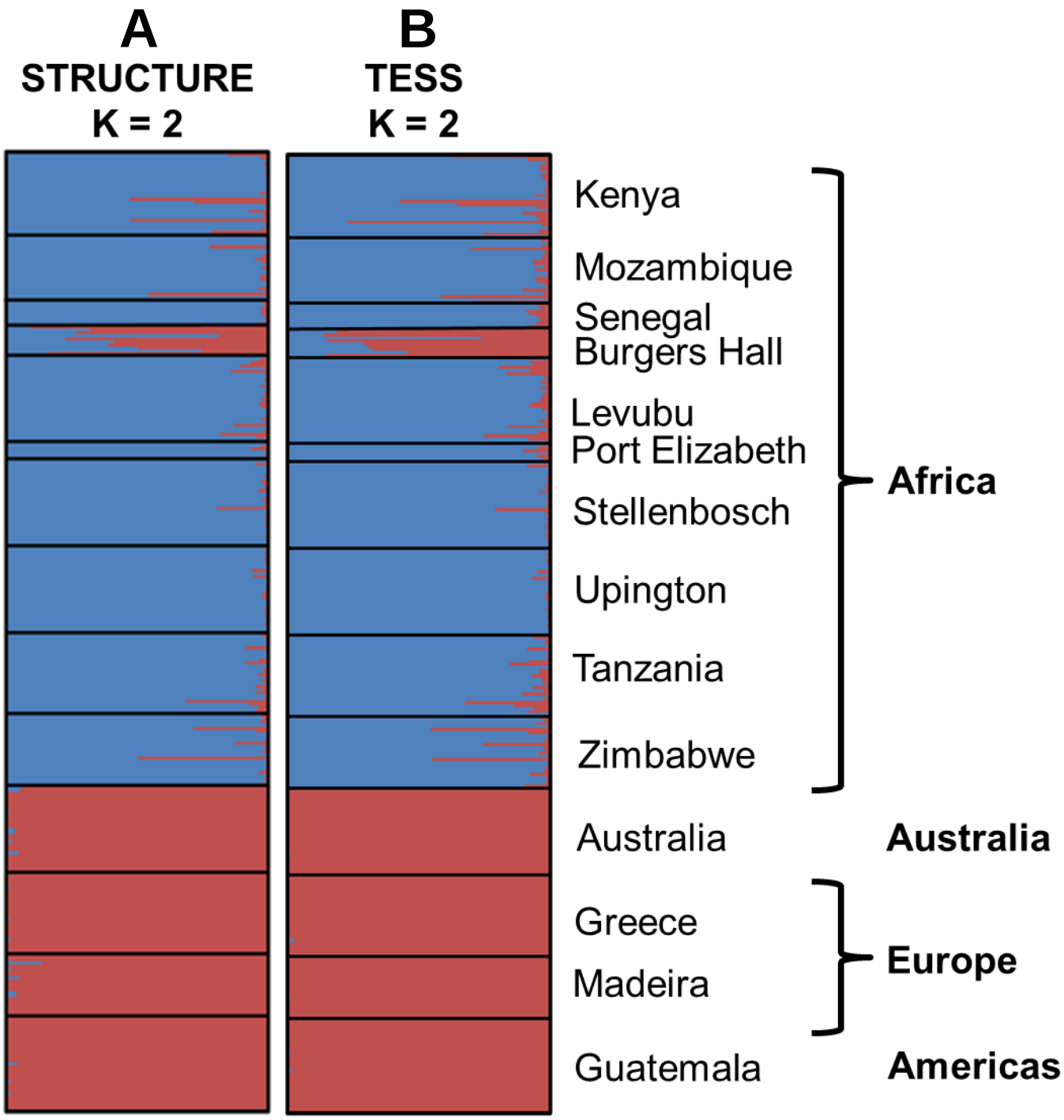


Figure 3.3 Assignment results from (A) STRUCTURE and (B) TESS for K=2 for 14 *Ceratitis capitata* populations.

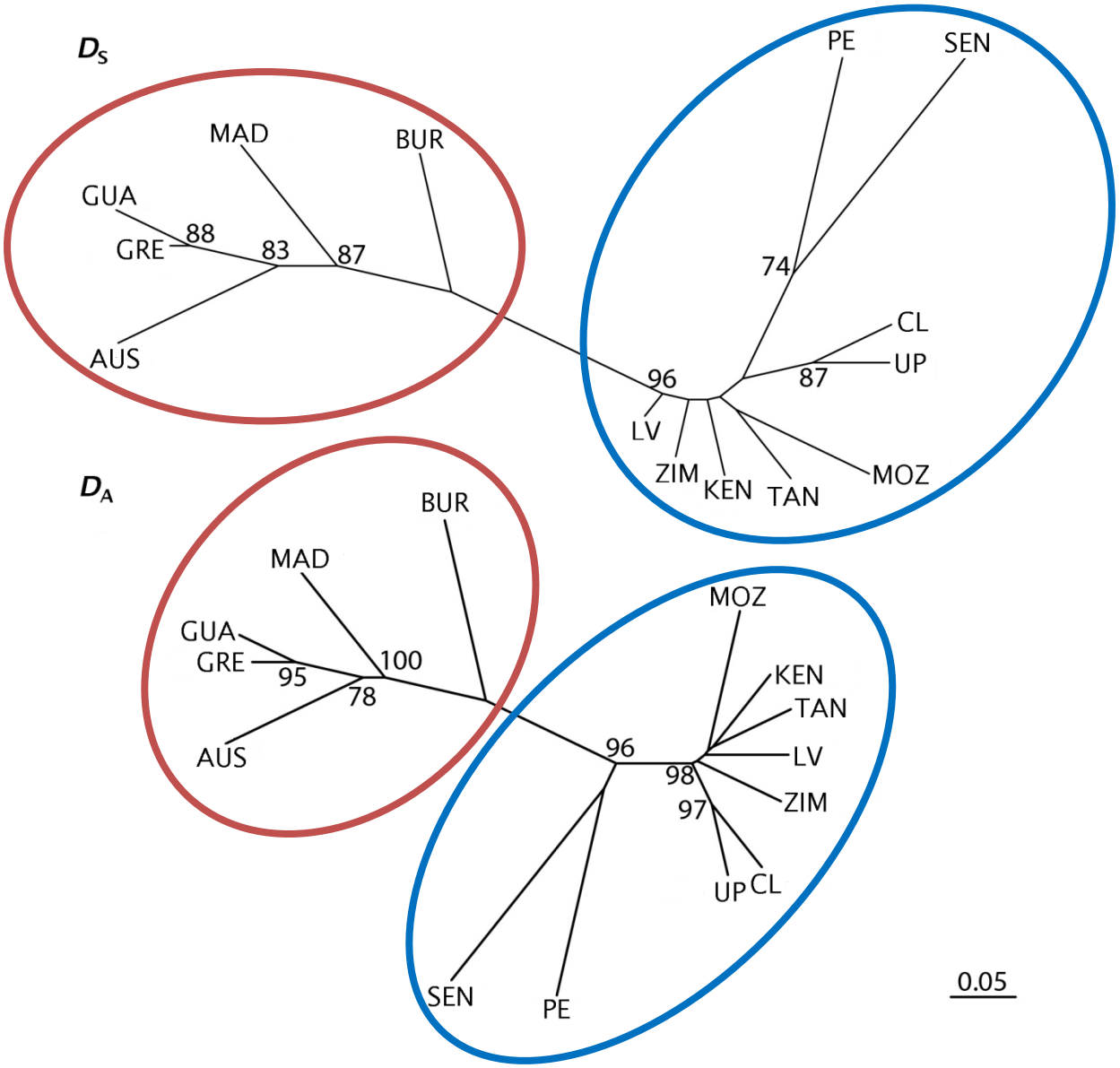


Figure 3.4 Unrooted nieghbour-joining trees for genetic distance based on shared alleles (D_S) and Nei's genetic distance (D_A). The number at each node indicates the bootstrap values after 10 000 bootstrap replicates. Only bootstrap values above 70% are shown.

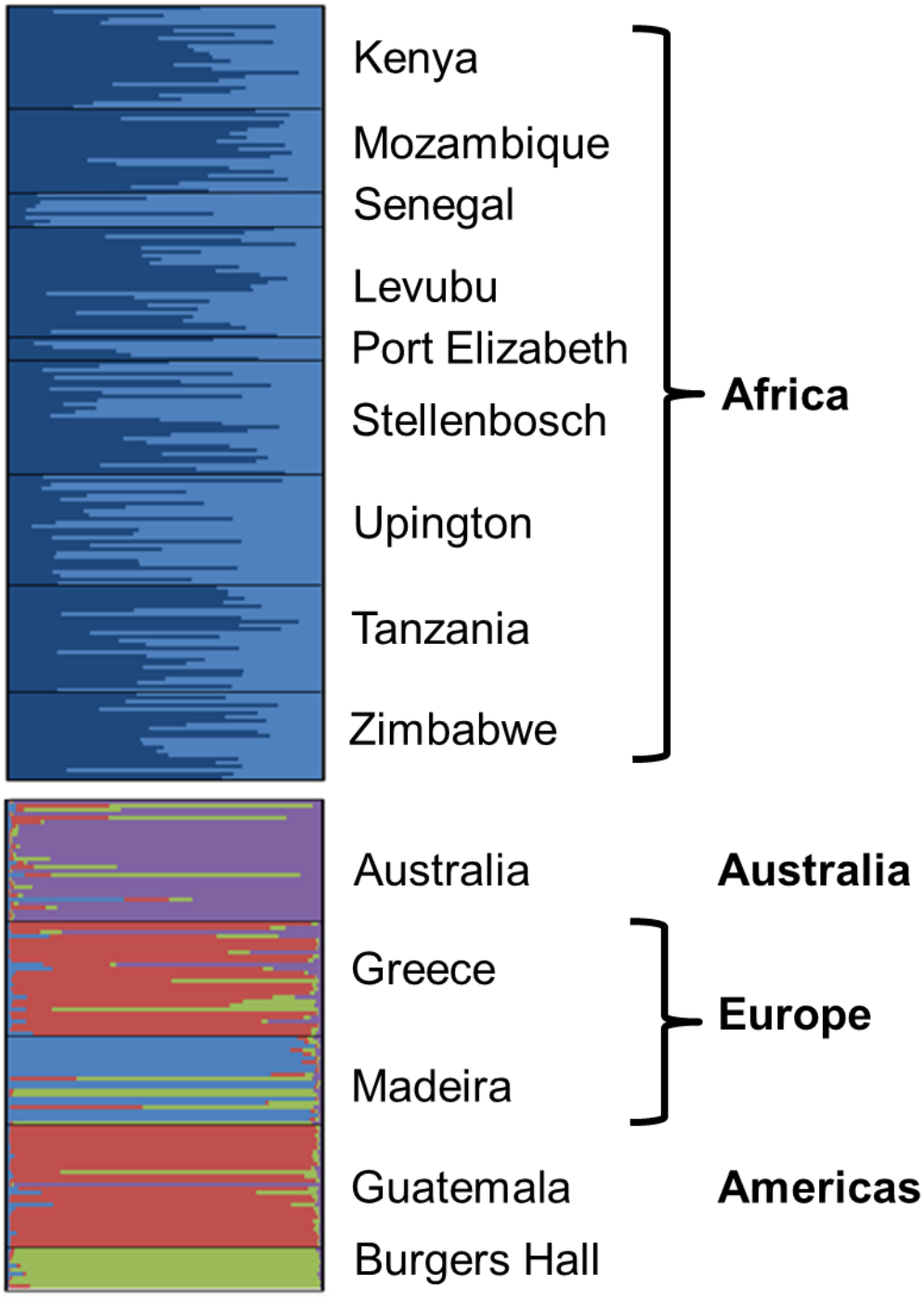


Figure 3.5 Assignment results from STRUCTURE for the (A) African cluster at K=2 and the (B) World cluster at *K*=4.

Table 3.5 Analysis of molecular variance (AMOVA) for 14 populations of *Ceratitis capitata* for five different scenarios tested.

Scenario	Among groups			Among populations within groups			Within populations		
	Va	Percentage	P	Vb	Percentage	P	Vc	Percentage	P
1	464.52	25.66	<0.0001	2.33	0.13	<0.0001	1343.42	74.21	0.015
2	615.37	30.23	<0.0001	77.03	3.78	<0.0001	1343.42	65.99	0.004
3	-35.12	-2.00	<0.0001	444.80	25.37	<0.0001	1343.42	76.63	0.464
4	-87.17	-5.02	<0.0001	479.03	27.61	<0.0001	1343.42	77.42	0.642
5	557.82	27.79	0.003	105.91	5.28	<0.0001	1343.42	66.93	<0.0001

Scenario 1: Subdivision by country (10 groups): South Africa (Burgershall, Levubu, Port Elizabeth, Stellenbosch, Upington) + Mozambique + Zimbabwe + Kenya + Senegal + Tanzania + Madeira + Greece + Guatemala + Australia

Scenario 2: Subdivision by geographical areas (4 groups): Africa (South Africa, Mozambique, Zimbabwe, Kenya, Senegal, Tanzania) + Europe (Greece, Madeira) + Americas (Guatemala) + Australia

Scenario 3: Genetic coancestry based on STRUCTURE (K=2): Africa *versus* the world (Europe, Americas, Australia)

Scenario 4: Genetic relationships from NJ tree (4 groups): [South Africa (Levubu, Stellenbosch, Upington), Mozambique, Zimbabwe, Kenya, Tanzania] + [Port Elizabeth, Senegal] + [Burgershall] + [Madeira, Greece, Guatemala, Australia]

Scenario 5: Subdivision based on historical colonization events: [South Africa, Mozambique, Zimbabwe, Kenya, Tanzania]+[Senegal]+[Madeira, Greece, Australia]+[Guatemala]

3.3.3 Demographic inference

Results from GENECLASS v2.0 indicated high levels of self-assignment of individuals to their resident populations (i.e. trapping location) with probability values (on the diagonal) ranging between 0.955 for Burgers Hall to 0.727 for Upington (Table 3.6). Southern and eastern Africa had the highest average assignment of individuals from a population to all the others (values in columns), with Zimbabwe having the highest contribution (0.452±0.163). Moreover, migration rates from other populations into a population (values in rows) were the highest for Greece (0.427±0.256). As expected, many African populations (Kenya, Levubu, Mozambique, Stellenbosch, Tanzania, Zimbabwe) which form part of the native range of *C. capitata* shared their genetic material (based on microsatellites) with all other sampled locations this is especially true for those locations in the introduced range.

3.3.4 Invasion pathway scenarios from ABC

To investigate the routes of colonization, four different sets of scenarios were tested using Approximate Bayesian Computations. In Table 3.6 results indicate that one scenario (Test 1, Scenario 2), based on posterior probability values and were clearly more supported than all other scenarios tested (Fig. 3.6). This scenario closely matched the dates of colonization available for *C. capitata* as well as the proposed historical routes of invasion (Fig. 3.7) with an initial colonization of Europe from Africa, a secondary colonization of Australia from Europe and finally, the colonization of the Americas from Africa.

Table 3.6 Average rate of assignment (m) of *Ceratitis capitata* individuals into (rows) and from (columns) each of the 14 sampling locations calculated in GENECLASS v2 (Piry *et al*. 2004).

	AUS	BUR	GRE	GUA	KEN	LV	MAD	MOZ	PE	SEN	CL	TAN	UP	ZIM	Ave.	SD
Australia	**0.807**	0.038	0.072	0.062	**0.433**	**0.515**	**0.253**	**0.410**	0.076	0.002	**0.179**	**0.333**	**0.122**	**0.508**	**0.272**	0.237
Burgers Hall	0.019	**0.955**	0.011	0.012	**0.371**	**0.664**	0.092	**0.648**	**0.175**	0.042	**0.438**	**0.365**	**0.300**	**0.563**	**0.332**	0.296
Greece	**0.145**	**0.212**	**0.757**	**0.447**	**0.643**	**0.641**	**0.484**	**0.671**	0.055	0.006	**0.411**	**0.618**	**0.210**	**0.677**	**0.427**	0.256
Guatemala	0.070	0.073	**0.265**	**0.764**	**0.517**	**0.585**	**0.334**	**0.699**	0.024	0.002	**0.248**	**0.485**	**0.112**	**0.504**	**0.335**	0.259
Kenya	0.000	0.000	0.000	0.000	**0.787**	**0.312**	0.000	**0.297**	0.038	0.001	**0.189**	**0.302**	**0.122**	**0.309**	**0.168**	0.223
Levubu	0.000	0.000	0.000	0.000	**0.299**	**0.788**	0.000	**0.299**	0.053	0.002	**0.194**	**0.349**	**0.131**	**0.427**	**0.182**	0.232
Madeira	0.022	0.051	0.025	0.032	**0.296**	**0.296**	**0.835**	**0.405**	0.034	0.005	**0.170**	**0.453**	**0.155**	**0.318**	**0.221**	0.235
Mozambique	0.000	0.000	0.000	0.000	**0.237**	**0.265**	0.000	**0.910**	0.041	0.000	**0.148**	**0.267**	0.087	**0.292**	**0.161**	0.245
Port Elizabeth	0.000	0.000	0.000	0.000	**0.226**	**0.185**	0.000	**0.149**	**0.828**	0.004	**0.319**	**0.269**	**0.125**	**0.340**	**0.175**	0.227
Senegal	0.000	0.000	0.000	0.000	**0.381**	**0.470**	0.000	**0.359**	**0.156**	**0.765**	**0.368**	**0.504**	**0.197**	**0.365**	**0.255**	0.242
Stellenbosch	0.000	0.000	0.000	0.000	**0.265**	**0.194**	0.000	**0.147**	0.033	0.001	**0.747**	**0.240**	**0.269**	**0.348**	**0.160**	0.211
Tanzania	0.000	0.000	0.000	0.000	**0.312**	**0.260**	0.000	**0.286**	0.032	0.001	**0.128**	**0.772**	**0.115**	**0.351**	**0.161**	0.221
Upington	0.000	0.000	0.000	0.000	**0.383**	**0.203**	0.000	**0.210**	0.091	0.001	**0.489**	**0.359**	**0.727**	**0.460**	**0.209**	0.238
Zimbabwe	0.000	0.000	0.000	0.000	**0.271**	**0.358**	0.000	**0.265**	0.057	0.000	**0.208**	**0.296**	**0.122**	**0.866**	**0.175**	0.239
Average	0.076	0.095	0.081	0.094	**0.387**	**0.410**	**0.143**	**0.411**	**0.121**	0.059	**0.303**	**0.401**	**0.200**	**0.452**		
SD	0.214	0.254	0.207	0.226	0.163	0.199	0.253	0.232	0.209	0.203	0.173	0.151	0.164	0.163		

In bold are the *m* values over 0.100 and underlined is the proportion of individuals assigned to their source population.

Table 3.7 Results from three tests with nine different scenarios tested in DIYABC v2.03 (Cornuet *et al*. 2014). I indicate Type I and Type II error rates, as well as Posterior Probabilities with 95% confidence intervals for all simulated data sets.

Test	Scenario	True scenario	Type I error	Type II error	Posterior probability (95% CI)
1	Africa⇨Madeira; Africa⇨Greece; Greece+Madeira⇨Australia; Africa⇨Guatemala	1	0.000	0.004	0.0036 (0.0000, 0.0148)
	Africa⇨Madeira⇨Greece; Greece+Madeira⇨Australia; Africa⇨Guatemala	**2**	**0.004**	**0.000**	**0.9964 (0.9852, 1.0000)**
2	Africa⇨Unsampled⇨Madeira⇨Greece; Unsampled+Greece+Madeira⇨Australia; Africa⇨Guatemala	1	0.000	0.000	0.0001 (0.000, 0.0033)
	Africa⇨Unsampled⇨Madeira⇨GreeceðGuatemala;Unsampled+Greece+Madeira⇨Australia	**2**	**0.008**	**0.002**	**0.8599 (0.8398, 0.8799)**
	Africa⇨Unsampled⇨Greece⇨MadeiraðGuatemala;Unsampled+Greece+Madeira⇨ Australia	3	0.002	0.000	0.1401 (0.1200,0.1601)
3	Africa (except Senegal)⇨Madeira⇨Greece; Madeira⇨Australia; Greece⇨Guatemala; Africa⇨Senegal	1	0.610	0.654	0.0320 (0.0001, 0.0639)
	Africa (except Senegal)⇨Senegal⇨Madeira⇨Greece; Madeira⇨Australia; Greece⇨Guatemala	**2**	**0.214**	**0.068**	**0.8373 (0.6476, 1.0000)**
	Africa (except Senegal)⇨Madeira⇨Greece; Madeira+Greece⇨Australia; Africa (except Senegal)⇨Guatemala; Africa⇨Senegal	3	0.548	0.51	0.0678 (0.0000, 0.1499)
	Africa (except Senegal)⇨Senegal⇨Madeira⇨Greece; Madeira+Greece⇨Australia; Africa (except Senegal)⇨Guatemala	4	0.334	0.354	0.0629 (0.0000, 0.1384)
4	**Africa (except Senegal/ South Africa)⇨Madeira⇨Greece; Madeira+Greece⇨Australia; Africa⇨South Africa; Africa⇨Senegal; Africa⇨Guatemala**	**1**	**0.00**	**0.054**	**0.5329 (0.3376, 0.7282)**
	Africa (except Senegal/ South Africa)⇨Senegal⇨Madeira⇨Greece; Madeira+Greece⇨Australia; Africa⇨South Africa; Africa⇨Guatemala	2	0.974	0.012	0.0002 (0.0000, 0.2229)
	Africa (except Senegal/ South Africa)⇨South Africa⇨Madeira⇨Greece; Madeira+Greece⇨Australia; Africa⇨Senegal; Africa⇨Guatemala	3	0.040	0.014	0.4669 (0.2441, 0.6897)

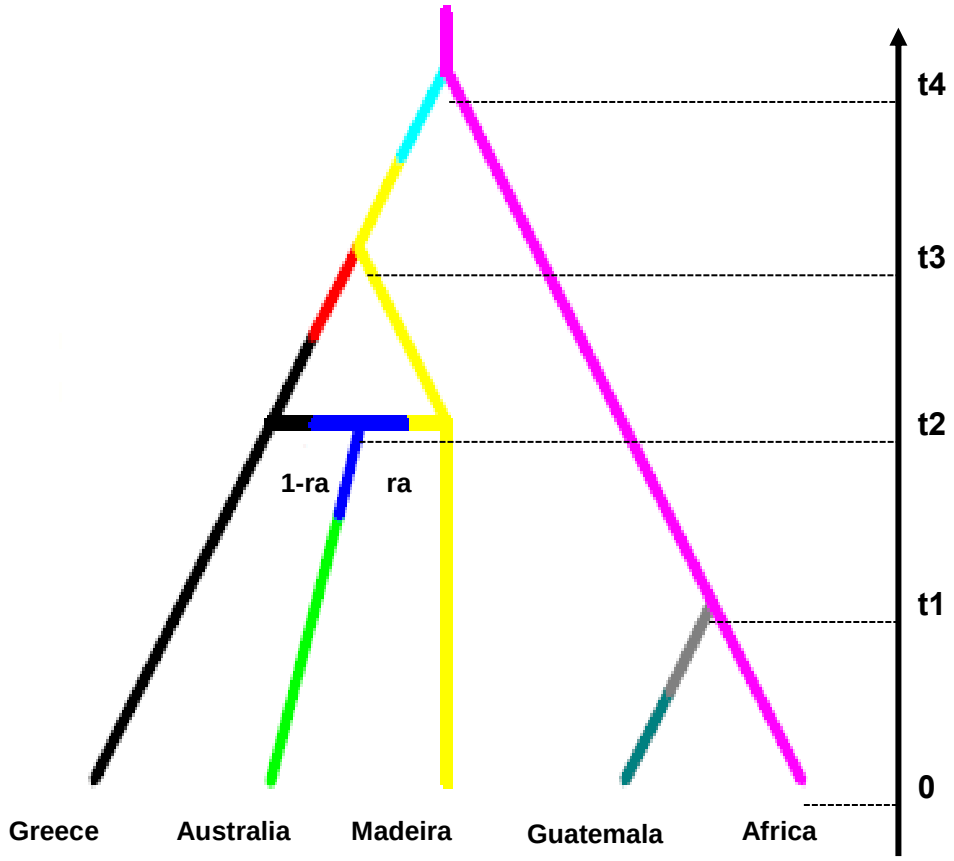


Figure 3.6 The most likely scenario (2) of Test 1 making use of Approximate Bayesian Computations (ABC) in DIYABC v2.03 (Cornuet *et al*. 2014). The timing of demographic events are indicated on the y-axis (not to scale). See a detailed description of this scenario in Table 3.7.

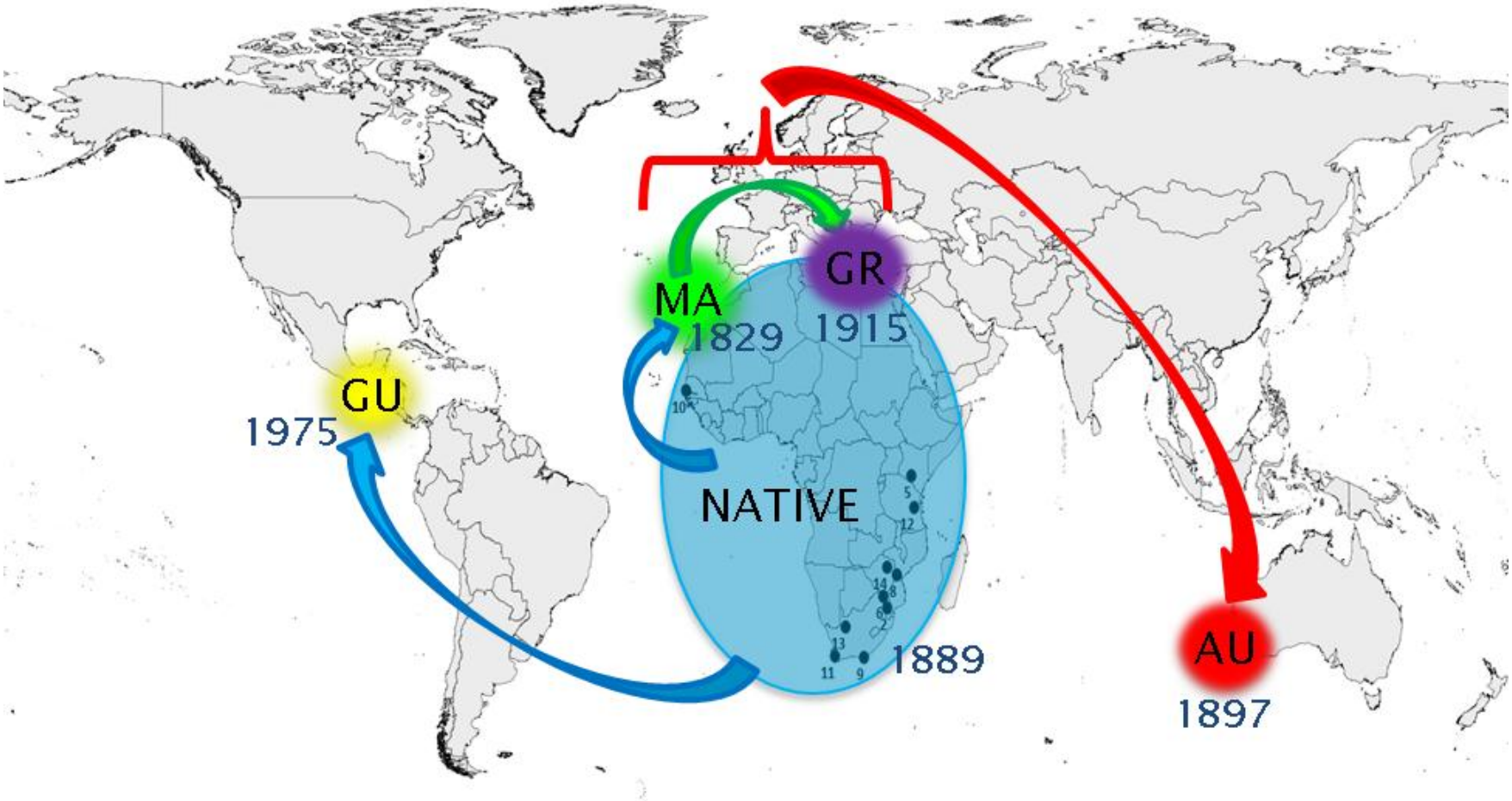


Figure 3.7 The route of invasion of *Ceratitis capitata*. Each coloured circle represents a sampling area in my data (GU= Guatemala, MA= Madeira, GR= Greece, AU= Australia, NATIVE= All African populations) and the bracket indicates admixture between populations. Arrows indicate the most likely scenario hypothesised (Table 3.7) and is supported by posterior probabilities and 95% confidence intervals (0.9964 [0.9852, 1.0000]). Dates indicated are those available as the earliest record.

3.4 Discussion

Global biological invasions, such as that of *Ceratitis capitata*, are typically characterized by reduced genetic diversity due to a small number of founders colonizing the introduced range. This creates a unique set of genetic and demographic attributes which were investigated here making use of 11 microsatellites for 14 multi-continent sampling locations of *C. capitata*. The colonization of *C. capitata* worldwide is well documented and results here match the set forth and tested hypothesis atleast those sampling localities included here.

The genetic diversity estimates from this research (expected heterozygosity, allelic richness, number of alleles and number of private alleles) are similar to those found in other studies done on *C. capitata* (Bonizzoni *et al*. 2001, 2004; Gasperi *et al*. 2002). I found high levels of genetic diversity (expected heterozygosity, number of private alleles, allelic richness) in all African populations, except Senegal and Burgers Hall, with a further decline in the genetic diversity estimates of populations in the introduced range (Australia, Greece, Guatemala, Madeira). The high level of genetic diversity found in this study for *C. capitata* in Africa is possibly due to their large effective population size. Both Senegal and Burgers Hall populations have diversity estimates similar to those of populations in the introduced range. As eastern and southern Africa has been identified as the native range of *C. capitata* (Gasperi *et al*. 2002; Malacrida *et al*.1998, 2007) it is perhaps unsurprising that Senegal (West Africa) has similar levels of genetic diversity as other populations in the introduced range. The diversity estimates for Senegal (H_E=0.588±0.136) are in fact higher than those of other locations in the introduced range which might possibly indicate that flies moved to Senegal from the native range via natural movement as well as by human assistance rather than only by jump dispersal. Interestingly, Burgers Hall, a location within South Africa, also has levels similar to that of populations in the introduced range, whilst other locations in South Africa seem to form part of the native range. These results are further supported by STRUCTURE, TESS and the Neighbour-joining trees which show that the Burgers Hall population groups closely with those from the introduced range (Australia, Greece, Guatemala, Madeira). Possible explanations for this may be an introduction event from the introduced range back into South Africa. All populations showed deviations from HWE and this can be attributed to inbreeding (F_{IS}=0.102-0.373), the Wahlund effect as well as null alleles. All populations sampled showed some null alleles which can arise because of their polymorphic genome (see e.g. Malacrida *et al*. 2007). These levels of inbreeding indicate that non-random mating is

occurring despite the high levels of gene flow and high effective population sizes and may well explain the observed deviations from HWE.

Furthermore, I found a clear pattern of genetic differentiation between the native African populations and those from the introduced range broadly forming two groups. The PCoA, STRUCTURE, TESS and NJ trees largely support the "out-of-Africa" hypothesis, grouping all the African populations together as well as all the introduced locations elsewhere in the world including Senegal. By contrast, almost all of the pairwise F_{ST} values were significant and those that were not were comparisons between African locations. The lack of differentiation throughout Africa indicates high levels of movement of *C. capitata* either by natural range expansion or via humans through corridors for movement supplied by the continuous trade of goods (formal and informal) or human travel. This high level of movement of flies is further aided by limited quarantine restriction within areas on the African continent that are not pest free. *Ceratitis capitata* is assumed to have travelled human-mediated to the Mediterranean coast (Maddison & Bartlett 1989) and the reduction in genetic diversity here seems to support the idea of a small number of founders introduced to this region. The European populations in my study group with Guatemala, although it has been proposed that Australia was a secondary colonization event from Europe (Hooper & Drew 1989), my data (STRUCTURE, TESS, NJ tree) to a certain extent indicate that the same may also be true for Guatemala. All populations considered had high levels of self-assignment to their source populations (Table 3.6), which is especially true for those populations in the introduced range. The most likely scenario chosen based on ABC calculations closely matches the proposed invasion pathway for *C. capitata* (Fig. 3.7). First, Madeira was colonized from the native range (Africa) and then *C. capitata* spread to Greece. These two locations formed an admixed population that secondarily colonized Australia. Finally Guatemala was colonized from the native range. To my knowledge this is the first time that the invasion pathway (albeit for limited number of sampling locations) of *C. capitata* has been tested and confirmed using ABC.

Inference of invasion pathways has traditionally been based on genetic distances and trees (Estoup & Guillemaud 2010). These methods are still widely used despite numerous drawbacks. One of these drawbacks is short divergence times which causes a lack of population differentiation due to other processes including drift and mutation as well as population bottlenecks. An additional drawback is multiple introductions from the same

source population and therefore the newly introduced populations are grouped together in the tree. Finally when using these genetic distance methods it is impossible to distinguish between the source and the introduction if both populations fall on terminal branches in the tree. Recently, clustering or assignment analyses have also been used to investigate invasion pathways, these include for example STRUCTURE (Pritchard *et al*. 2000) and BAPS (Corander *et al*. 2004). If the introduced population distinctly clusters with a source populations it is considered as the origin of the introduced population. These inferences are however difficult to make and some drawbacks here include unsampled source populations as well as small numbers of markers used in the analysis. All of these methods share a common limitation in which stochastic and demographic parameters are not sufficiently integrated (Estoup & Guillemaud 2010).

The ABC method on the other hand has some major advantages as it uses a large amount of data (genetic and demographic) simultaneously, it allows for multiple competing scenarios (Guillemaud *et al*. 2010) and the estimation of probability values with confidence intervals (Cornuet *et al*. 2008) to choose the best scenario. One of the most important advantages is the ability to model ghost populations, which are unsampled but that does contribute to the invasion process. This method also comes with its drawbacks and as with other methods the main limitation here is that a large number of sites need to be sampled in both the introduced and native range and analysed with sufficiently large numbers of markers to confidently obtain a true reflection of the invasion pathway of the species in question (Estoup & Guillemaud 2010).

Previous studies investigating the invasion pathway of *C. capitata* showed decreased genetic variability at two scales from Africa to the Mediterranean Basin as well as from Latin America to the Pacific (Gomulski *et al*. 1998; Malacrida *et al*. 1998; Gasperi *et al*. 2002; Bonizzoni *et al*. 2000) but are limited by what we can learn from genetic distance. Subsequent studies looking at macrogeographic scales and trying to identify source populations and therefore indirectly invasion pathways were based on genetic similarity and therefore suffered from some of the above mentioned limitations (Bonizzoni *et al*. 2001, 2004). Here the different *C. capitata* invasion hypotheses could be statistically tested and compared and the most likely scenario chosen of the invasion routes of the locations included here.

There are low levels of connectivity between the African continent and the introduced range although high levels of connectivity exist on a regional scale. This information in turn is important for the management of *C. capitata* worldwide. The lack of connectivity indicates that quarantine measures for export consignments from Africa are successful in limiting the movement of fruit flies inter-continentally. However, there is evidence for high levels of movement of fruit flies on the African continent. This does not bode well for the fruit industry especially in the light of preventing new invasions into a region. A case in point is that of *Bactrocera invadens* (*B. dorsalis*) which after its introduction to the African continent in 2003 (Lux *et al*. 2003) has spread over large parts of the continent despite quarantine and eradication efforts (De Meyer *et al*. 2010). Information from the reconstruction of the routes of invasion for *C. capitata* is important to understand the different evolutionary and environmental factors that influence successful invasions that can be incorporated into strategies for the control and prevention of new invasions (Estoup & Guillemaud 2010). Future studies should therefore focus on including more samples from native and introduced locations for *C. capitata* as well as higher numbers of molecular markers to infer a more global invasion pathway.

CHAPTER 4: A LACK OF GENETIC STRUCTURE IN SOUTH AFRICA OF THE AGRICULTURAL PEST FRUIT FLY, *CERATITIS ROSA*, INDICATING HIGH DISPERSAL POTENTIAL

4.1 Introduction

The fruit flies within the Tephritidae (Diptera) constitute a group of agricultural pests with worldwide importance as they attack a broad range of commercial crops (White & Elson-Harris 1994). Two of the most economically significant species in Africa are members of the genus *Ceratitis* (White & Elson-Harris 1994) the Mediterranean fruit fly (*Ceratitis capitata* (Weidemann)) and the Natal fruit fly (*Ceratitis rosa* Karsch). Unlike *C. capitata* that has become invasive in many locations outside of its native range, *C. rosa* is largely restricted to eastern and southern Africa, but has subsequently spread to the Indian Ocean islands of La Réunion and Mauritius and outcompeted *C. capitata* in some instances (White *et al*. 2001; Duyck *et al*. 2006; De Meyer *et al*. 2008). Since the life-history characteristics and thermal niche of *C. rosa* is similar to that of *C. capitata* (Duyck & Quilici 2002; De Meyer *et al*. 2008), it raises major concerns to the fruit industry worldwide. These concerns are further underscored due to *C. rosa*'s wide host range and polyphagous nature (White & Elson-Harris 1994; De Meyer *et al*. 2002). *Ceratitis rosa* therefore poses a high risk of establishment outside of its native range (De Meyer *et al*. 2008; De Villiers *et al*. 2013b).

Despite the importance of *C. rosa* as a major agricultural pest and the generally high concerns for further establishment and spread within and outside of Africa, the population genetics of *C. rosa* have generally been poorly studied. However, some early work which did include this species reported some differentiation between the African mainland and island populations of Mauritius and La Réunion (Baliraine *et al*. 2004). Primarily research undertaken on *C. rosa* to date has focused mainly on molecular diagnostics (Douglas & Haymer 2001; Barr *et al*. 2006), taxonomic status and phylogenetic relationships (Barr & McPheron 2006; Virgilio *et al*. 2008). *Ceratitis rosa* forms part of a complex of three closely-related fruit flies called the *Ceratitis* FAR complex (Diptera: Tephritidae) which also includes *Ceratitis fasciventris* (Bezzi) and *Ceratitis anonae* Graham (Virgilio *et al*. 2008). Five genotypic clusters characterize these three species with inter-cluster divergences being equal to or higher than divergences between recognized species (Virgilio *et al*. 2013). Within *C. rosa*, two genotypic clusters (morphotypes) were identified (as R1 and R2) with largely overlapping geographic ranges (Virgilio *et al*. 2013); little to no genetic differentiation exists within each morphotype. The genetic differences between the morphotypes are partly supported by morphological differences in the shape and feathering of the mid tibia of the males with no definite distinguishing characters identified for the females as yet (Virgilio *et al*. 2013).

South Africa is a major producer and exporter of deciduous fruit with approximately 76 000 ha under production with annual revenue export earnings of *c*. US$450 million (Barnes *et al.* 2002; Barnes & Venter 2006). A better understanding of the distribution of the two morphotypes within South Africa as well as their regional scale population structure is critical, which in turn provides essential information required for Integrated Pest Management (IPM) strategies. In South Africa, a unique situation exists as the two morphotypes (R1 and R2) occur sympatrically in the northern regions of the country (See Fig. 3 in Virgilio *et al.* 2013). Moreover, morphological inspection of the legs of *C. rosa* males from South Africa indicated additional locations at which both morphotypes (albeit R1 at low abundance) occur sympatrically in the north of South Africa, especially the Mpumalanga and Limpopo provinces (Tzaneen, Rustenburg, Komatiepoort, Nelspruit, Marblehall) (M. de Meyer, personal communication). However, given the small number of locations included for South African *C. rosa* in the Virgilio *et al*. (2013) study, the extent of overlap as well as distribution of each cluster remains largely unknown. To address this, I generated both molecular and morphological data for the same individuals resulting in two parallel, but independent data sets. Surprisingly few studies to date have adopted this approach in spite of the clear potential (but see Bouyer *et al*. 2007; Francoy *et al*. 2009; Bomfim *et al*. 2011; Schutze *et al*. 2012a; Krosch *et al*. 2013).

Microsatellite markers are regularly used to document genetic structure in tephritids (e.g. Bonizzoni *et al*. 2001, 2004; Baliraine *et al*. 2004; Nardi *et al*. 2005; Gilchrist & Meats 2009; Aketarawong *et al*. 2014). Molecular information provides indirect estimates of dispersal (gene flow) between populations. The advantage of such an approach is that estimates are averaged over several generations, overcoming some of the drawbacks of using more direct measurements (such as mark-and-recapture methods) (Bohonak 1999). Comparisons between direct measures of migration and indirect measures often provide different estimates, with gene flow estimates based on DNA data typically being higher (Bohonak 1999). This discrepancy is very clear in *C. capitata* where direct measures of life-time migration distances are, on average, between 0.5 and 9.5 km (Meats & Smallridge 2007) and reported estimates based on indirect measures are in the order of hundreds of kilometers (e.g. Karsten *et al*. 2013). Studies on the maximum flight distance of *C. rosa* is deficient, but based on the leptokurtic dispersal of tephritids in general the distances for *C. rosa* should be in the same order of magnitude as that of *C. capitata*. In combination with molecular information, I also include geometric morphometric measurements, which have been shown to be effective in discriminating groups at intra- and inter-specific levels (Gilchrist & Crisafulli 2006; Bouyer *et*

al. 2007; Marsteller *et al*. 2009). Geometric morphometrics quantifies shape variation between homologous structures (e.g. landmarks on wings) and then compares this shape data between groups or individuals (Rohlf & Marcus 1993; Rohlf 1999). Furthermore, morphological data may be amongst the first characters to show differences between populations (Bouyer *et al*. 2007).

The broad aims of this study are therefore to: (1) determine the distribution of the *C. rosa* R1 and R2 genotypic clusters across South Africa with possible regions of overlap, (2) determine the phylogeographic patterns for these two genotypic clusters including possible evolutionary breaks, and (3) determine the levels of connectivity (gene flow) between populations. I hypothesized that the majority of localities across South Africa will have only one genotypic cluster, but that there may be areas of overlap for the two clusters in the northern regions of the country. Furthermore, these areas of overlap will provide estimates of the movement of individuals between different sites. Here I make use of both morphological and molecular markers to identify the pattern of population structuring of the two morphotypes within South Africa and to investigate the connectedness of pest-occupied sites making use of both morphological and molecular (microsatellite) markers, with the ultimate aim of fine-tuning area-wide pest management recommendations.

4.2 Materials and Methods

4.2.1 Sampling sites and fly collection

Ceratitis rosa individuals were collected from 22 sites throughout South Africa (Fig. 4.1; Table 4.1) using Bucket traps (Chempac, Paarl, South Africa) baited with the three-component attractant, Biolure® (Chempac, Paarl, South Africa). Flies from different traps were handled separately and were sexed and identified using a stereomicroscope. After identification, both wings (left and right) were removed for morphological analyses and DNA was extracted from the bodies using a DNeasy® tissue kit (QIAGEN Inc.). Following DNA extractions, exoskeletons were washed and stored in absolute ethanol for morphotype identification.

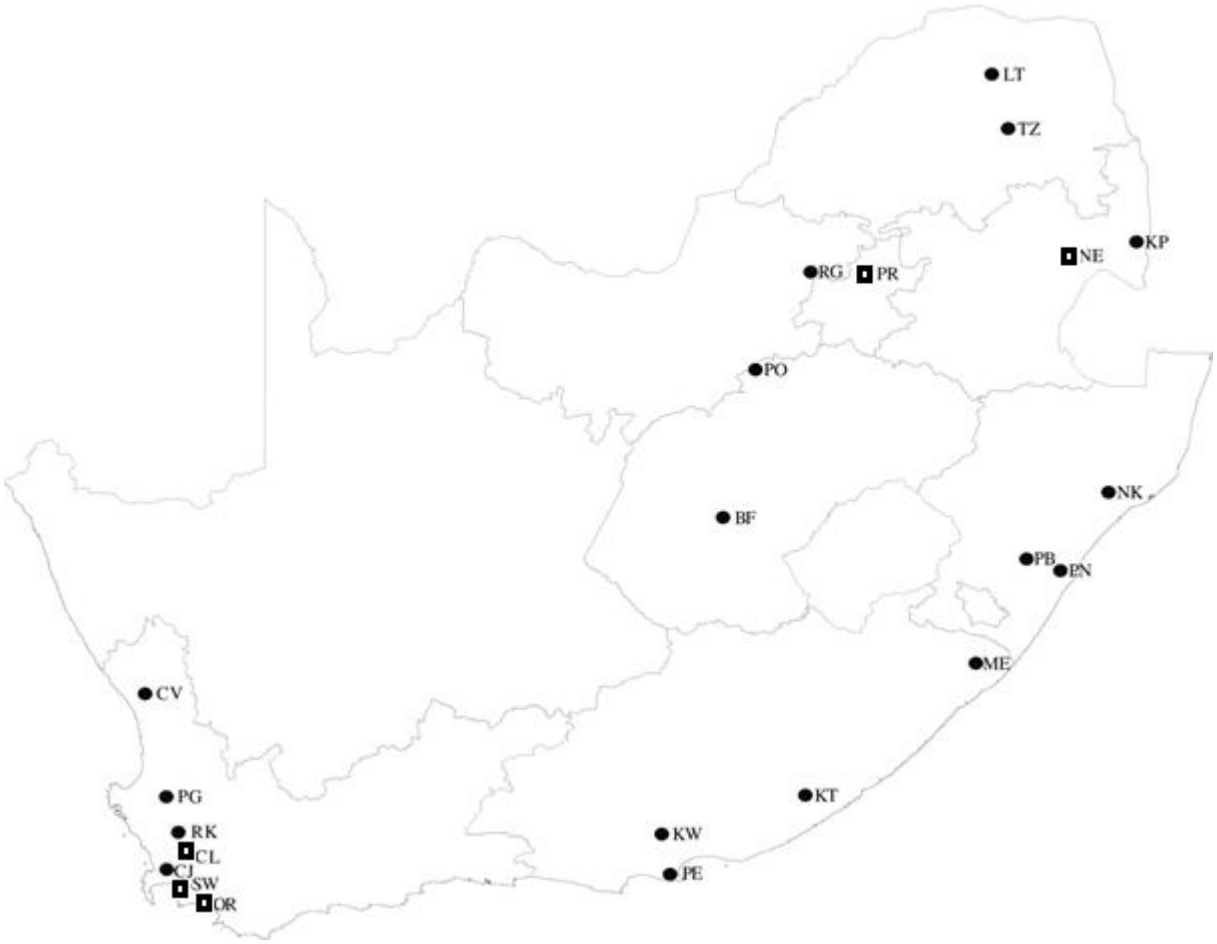


Figure 4.1 The 22 sampling sites for *Ceratitis rosa* in South Africa. The five square locations correspond to overlapping sampling localities with Virgilio *et al*. 2013. Population locality names are given in full in Table 4.1.

Table 4.1 The locations of *Ceratitis rosa* sampling in South Africa with sample size (N), number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity (± standard error), the inbreeding coefficient (F_{IS}) and the mean null allele frequency (A_n; Dempster *et al*. 1977; SD in parentheses).

Location	ID	GPS coordinates		N (genetics/wings)	N_A	H_E	H_O	F_{IS}	A_n
		Latitude	Longitude						
Bloemfontein	BF	-29.09	26.280	27/12	6.545	0.636±0.168	0.548±0.176	0.159	0.064 (0.088)
King Williams Town	KT	-32.87	27.391	14/11	5.727	0.611±0.174	0.509±0.237	0.206	0.080 (0.098)
Kirkwood	KW	-33.4	25.440	28/13	6.273	0.607±0.189	0.520±0.235	0.163	0.071 (0.116)
Komatiepoort	KP	-25.35	31.914	28/12	7.364	0.654±0.133	0.567±0.169	0.155	0.070 (0.082)
Louis Trichardt	LT	-23.07	29.930	16/12	5.364	0.642±0.119	0.570±0.235	0.146	0.061 (0.111)
Lutzville	CV	-31.56	18.359	10/6	4.364	0.597±0.171	0.510±0.256	0.203	0.080 (0.127)
Mt. Edgecombe	ME	-31.08	29.722	5/5	3.818	0.607±0.132	0.582±0.244	0.152	0.066 (0.085)
Nelspruit	NE	-25.53	30.991	30/21	6.909	0.660±0.096	0.591±0.172	0.122	0.062 (0.075)
Nkwalini	NK	-28.75	31.524	8/10	4.091	0.577±0.193	0.519±0.270	0.176	0.071 (0.121)
Onrus River	OR	-34.41	19.172	23/12	5.818	0.645±0.125	0.547±0.165	0.176	0.061 (0.082)
Paarl	CJ	-33.73	18.962	26/15	5.455	0.622±0.131	0.517±0.170	0.189	0.081 (0.094)
Pietermaritzburg	PB	-29.66	30.402	28/15	6.455	0.643±0.110	0.536±0.192	0.185	0.068 (0.088)
Piketberg	PG	-32.9	18.751	23/8	5.364	0.640±0.115	0.510±0.188	0.227	0.084 (0.094)
Pinetown	PN	-29.82	30.871	10/7	4.909	0.689±0.140	0.532±0.188	0.284	0.101 (0.098)
Port Elizabeth	PE	-33.96	25.571	28/16	7.727	0.698±0.115	0.600±0.154	0.160	0.057 (0.098)
Potchefstroom	PO	-27.08	26.723	12/10	5.364	0.624±0.153	0.540±0.206	0.179	0.065 (0.108)
Pretoria	PR	-25.78	28.244	23/17	6.727	0.662±0.148	0.557±0.198	0.183	0.065 (0.105)
Riebeeck Kasteel	RK	-33.39	18.891	18/6	4.545	0.588±0.182	0.416±0.215	0.323	0.111 (0.102)
Rustenburg	RG	-25.76	27.474	27/8	6.909	0.661±0.116	0.544±0.140	0.197	0.077 (0.087)
Somerset West	SW	-34.06	18.862	28/15	5.818	0.643±0.127	0.514±0.219	0.220	0.083 (0.120)
Stellenbosch	CL	-33.88	18.741	25/25	5.909	0.640±0.152	0.448±0.202	0.321	0.118 (0.116)
Tzaneen	TZ	-23.81	30.158	23/10	6.818	0.689±0.140	0.531±0.188	0.158	0.072 (0.070)

4.2.2 Microsatellite genotyping and analysis

All individuals collected (N= 458) were genotyped for 12 microsatellite markers (Delatte *et al*. 2013). A PCR reaction consisting of 2 µL of DNA (*c.* 30 ng), 2 µL of primer mix (2 mM), 6 µL of 2x QIAGEN Multiplex Master Mix and 1 µL water (Multiplex PCR kit QIAGEN Inc.) were set up for each individual. PCR conditions followed Delatte *et al*. (2013). For each plate a positive control (individuals genotyped in each plate) was included to check for amplification consistency when reading plates. Samples were genotyped on an ABI 3130 Automated Sequencer (Applied Biosystems, Foster City, California, USA) and the alleles scored using GENEMAPPER v3.7 software (Applied Biosystems, Foster City, California, USA).

Tests for linkage disequilibrium (LD) and departures from Hardy-Weinberg equilibrium (HWE) were conducted in GENEPOP v4.0.10 and statistical significance determined through 1 000 permutations (Raymond & Rousset 1995; Rousset 2008). Levels of significance were adjusted using False Discovery Rates (QVALUE; Storey 2002). Linkage disequilibrium was detected for one of the markers (FAR9 with FAR3 and FAR15) and FAR9 was therefore excluded from subsequent analyses. To test for the presence of null alleles (A_n) their frequencies in each of the populations for each of the loci was estimated in FREENA v1.0 (Chapuis & Estoup 2007) using the algorithm from Dempster *et al*. (1977). For each locus and for each site I calculated expected heterozygosity (H_E, the expected allele frequencies under HWE), observed heterozygosity (H_O, the actual heterozygosity in a population measured), the number of alleles (N_A) and the inbreeding coefficient (F_{IS}, the proportion of variance from a population that is contained in an individual) (GENETIX v4.05.2; Belkhir *et al*. 1996-2004; GenAlEx v6.5; Peakall & Smouse 2006, 2012).

Bottleneck v1.2.0.2 (Cornuet & Luikart 1996) was used to investigate changes in population sizes. Using both the two-phase model of mutation (TPM) and the stepwise mutation model (SMM) I computed deviations from expected heterozygosity through 10 000 permutations. To determine whether a population is characterized by a heterozygosity excess or deficit I made use of one-tailed Wilcoxon signed-rank tests. The hypothesis of isolation by distance (IBD) was assessed by the linear correlation of geographic distance (ln distance in km) with genetic distance (F_{ST}/ (1-F_{ST})) (GENEPOP v4.0.10; Raymond & Rousset 1995; Rousset 2008).

Population differentiation was assessed using the Bayesian clustering method implemented in the program STRUCTURE v2.3.4 (Pritchard *et al*. 2000; Falush *et al*. 2003) as well as

estimates of pairwise F_{ST} values in MICROSATELLITE ANALYSER v4.05 (MSA; Dieringer & Schlötterer 2003). Multilocus genotypes were assigned in STRUCTURE without including prior spatial population information. I ran STRUCTURE for 3 000 000 MCMC permutations (where statistical parameters reached stability) with 1 500 000 runs discarded as burn-in from each run for 5 independent runs for each *K* value, varying respectively between 1 and 22 (the maximum number of sampled sites). I implemented the admixture model with correlated allele frequencies, as I expect common ancestry, and I allowed the Dirichlet distribution of allelic frequencies (λ) to be inferred separately for each site. Choosing the optimal number for *K* is up to the user based on the data. Inspecting the log-probabilities of several values of *K* is thought to be a good estimator of the true value of *K* (Pritchard *et al*. 2000). This method can, however, be problematic when there is more than one high value of Ln P(X/*K*) and I therefore also inspected the delta *K* value calculated according to Evanno *et al*. (2005) implemented in the online resource STRUCTURE HARVESTER (Earl & Von Holdt 2012). This method also has some drawbacks, more specifically the inability to assess *K*=1 as the optimal number of clusters. Runs were averaged in CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007) and visualized in DISTRUCT v1.1 (Rosenberg 2004). The hierarchical structuring of populations were investigated using an AMOVA based on R_{ST} estimates in ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010) using 10 000 permutations.

4.2.3 Wing morphometrics

Wings (left and right) were permanently mounted on microscope slides using Entellan®, a rapid embedding agent. I chose fourteen homologous type I landmarks (Bookstein 1991) (Fig. 4.2). Twelve of the chosen landmarks were based on Schutze *et al*. (2012b); the remaining two were chosen to improve coverage of the wing. Each wing was imaged using a Leica MZ 16A microscope camera. The 14 landmarks were superimposed on the images of the wings and digitized in tpsDig version 2.17 (Rohlf 2005). To assess possible measurement errors in the data I investigated digitizing error (positioning of the landmarks) and imaging error (owing to taking the picture). For this purpose I constructed an additional dataset comprising a subset of the individuals from the complete data (henceforth known as the error dataset). For each individual, the wings were photographed twice (imaging error) and landmarks were placed on wings independently (digitizing error).

After acquisition of the x, y coordinates for each set of landmarks the data were imported into MorphoJ version 1.05c for shape analysis (Klingenberg 2011). In MorphoJ I performed a full Procrustes fit to extract shape information and ran a Generalised Procrustes ANOVA (GPA)

on both the error dataset and the complete dataset. A covariance matrix for the complete data was used for calculating a Principle Components Analysis (PCA) based on an averaged dataset (left and right wing), followed by a Canonical Variate Analysis (CVA). Finally, to test for IBD I regressed the Mahalanobis distance based on the shape variable (from the CVA) against geographic distance (km).

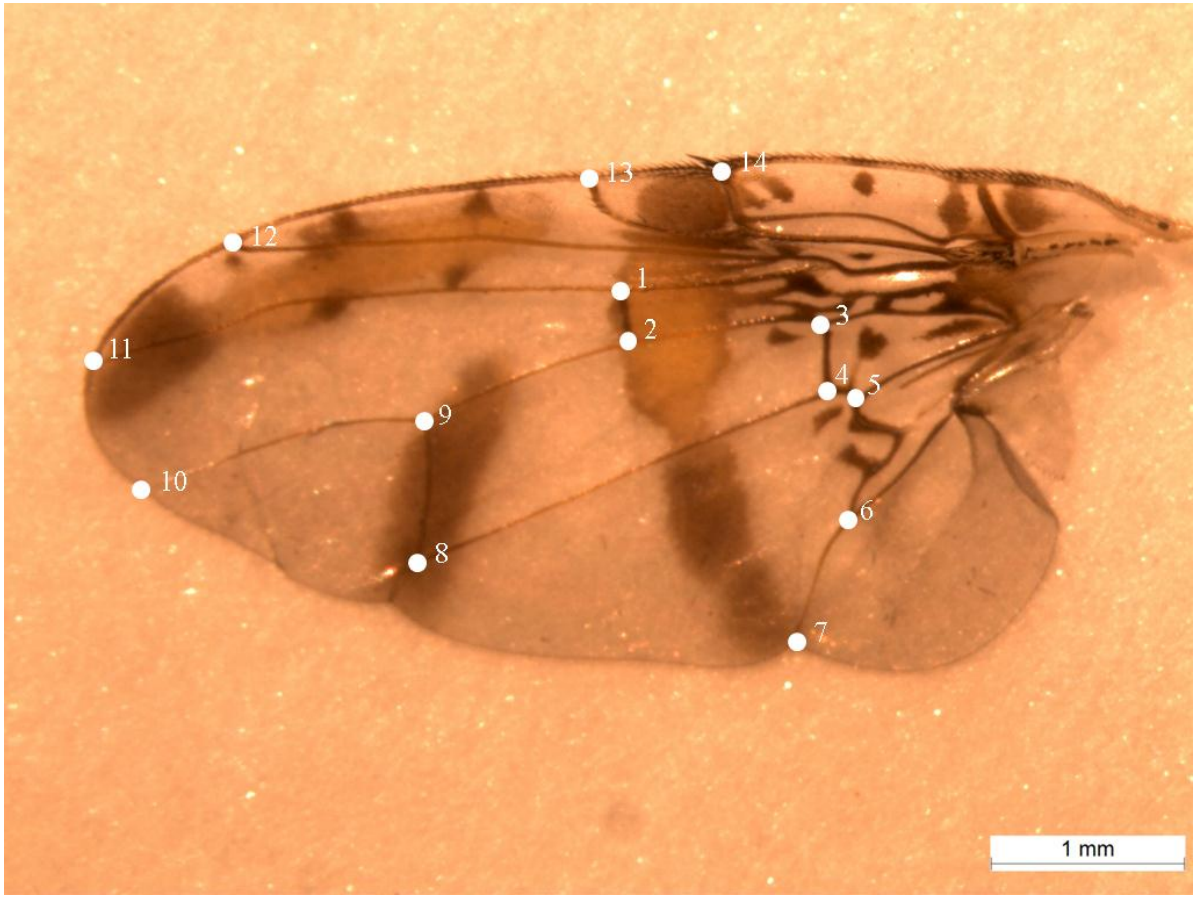


Figure 4.2 Right wing of *Ceratitis rosa* showing the fourteen type I landmarks (Bookstein 1991) used in the geometric morphometric data set. Scale = 1mm.

4.3 Results

4.3.1 Microsatellite DNA analyses

Analyses of the 11 polymorphic microsatellite markers in *C. rosa* for 22 geographic localities showed that all of the populations deviated from HWE. Inbreeding (F_{IS}=0.122-0.323) and an excess of homozygotes (Table 4.1) was evident within sites, indicative of non-random mating (also reported by Virgilio *et al*. 2013). The number of alleles (N_A) for the different loci varied, on average, between 3.81 and 7.73 (Table 4.1). The average null allele frequency per locus ranged between 0.010 (SD=0.017) at FAR14 and 0.272 (SD=0.073) at FAR1 (Table 4.1; Appendix IV). All loci were included in all analyses given that F_{ST} estimates and genetic distances are largely unbiased when population structure is absent (see later) (Chapuis & Estoup 2007).

Genetic diversity, as measured by mean expected heterozygosity (H_E), ranged between 0.577 (Nkwalini) and 0.698 (Port Elizabeth) (Table 4.1). Results from a one-tailed Wilcoxon signed rank test indicated no significant heterozygosity excess (indicative of recent bottlenecks) in all of the sites considered under both the TPM and SMM models. There was, however, some indication of population expansion in some of the sites under both these models (Table 4.2).

Tests for isolation by distance (IBD) showed no correlation between genetic distance and geographic distance (r=0.088, p=0.184; Fig. 4.3). Results from the Bayesian clustering of individuals showed a peak in the $\triangle K$ at K=2 (Fig. 4.4 (A)). Taking into account that the approach from Evanno and co-workers (2005) cannot account for K=1, I also examined the posterior probabilities (Ln P(X/K) which was highest for K=1. The presence of only a single morphotype in South Africa was somewhat unexpected and contrary to what has previously been reported by Virgilio *et al*. (2013) although several localities included here was identical to those sampled for the 2013 study. This result was confirmed by STRUCTURE analyses including only those localities included in both the present and 2013 study (Fig. 4.4 (B)).

Table 4.2 The probabilities of one-tailed Wilcoxon signed-rank tests to validate significant heterozygosity excess or deficit expected in populations that experienced recent reductions or expansions in their population sizes.

	TPM		SMM	
Location	deficit	excess	deficit	excess
Bloemfontein	0.183	0.840	**0.006**	0.995
King Williams Town	0.051	0.959	**0.011**	0.992
Kirkwood	0.087	0.926	**0.003**	0.998
Komatiepoort	**0.003**	0.998	**0.000**	1.000
Louis Trichardt	0.160	0.861	**0.005**	0.997
Lutzville	0.618	0.416	0.183	0.840
Mt. Edgecombe	0.740	0.289	0.319	0.711
Nelspruit	**0.008**	0.994	**0.000**	1.000
Nkwalini	0.618	0.416	0.416	0.618
Onrus River	0.289	0.740	0.051	0.959
Paarl	0.207	0.817	**0.027**	0.990
Pietermaritzburg	**0.011**	0.992	**0.001**	0.999
Piketberg	0.382	0.650	**0.011**	0.992
Pinetown	0.949	0.062	0.681	0.350
Port Elizabeth	0.103	0.913	**0.005**	0.997
Potchefstroom	0.103	0.913	**0.006**	0.995
Pretoria	0.139	0.880	**0.011**	0.992
Riebeeck Kasteel	0.650	0.382	0.232	0.793
Rustenburg	0.160	0.861	**0.003**	0.998
Somerset West	0.289	0.740	**0.034**	0.973
Stellenbosch	0.232	0.793	**0.042**	0.966
Tzaneen	0.074	0.938	**0.011**	0.992

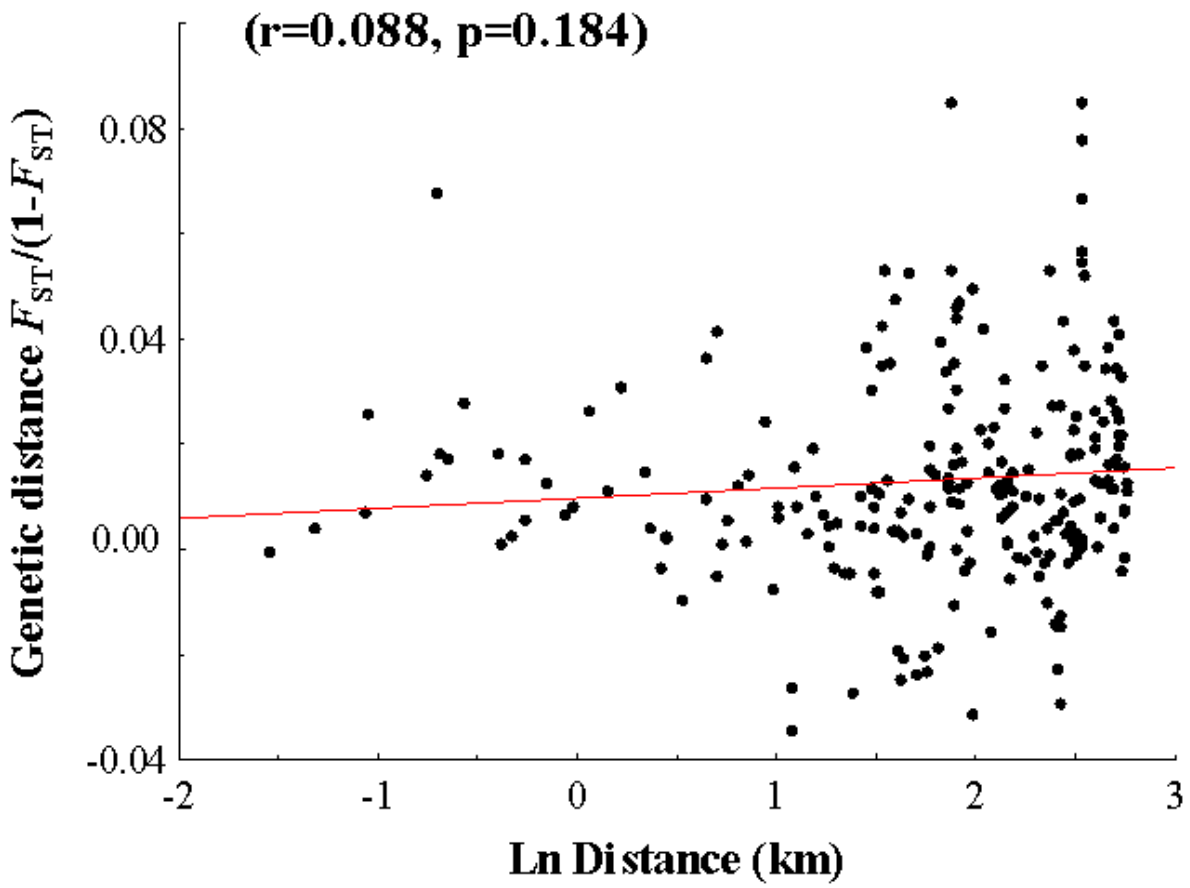


Figure 4.3 Linear correlation of genetic distance (pairwise $F_{ST}/1\text{-}F_{ST}$) against geographic distance (ln distance in km) between sampling locations.

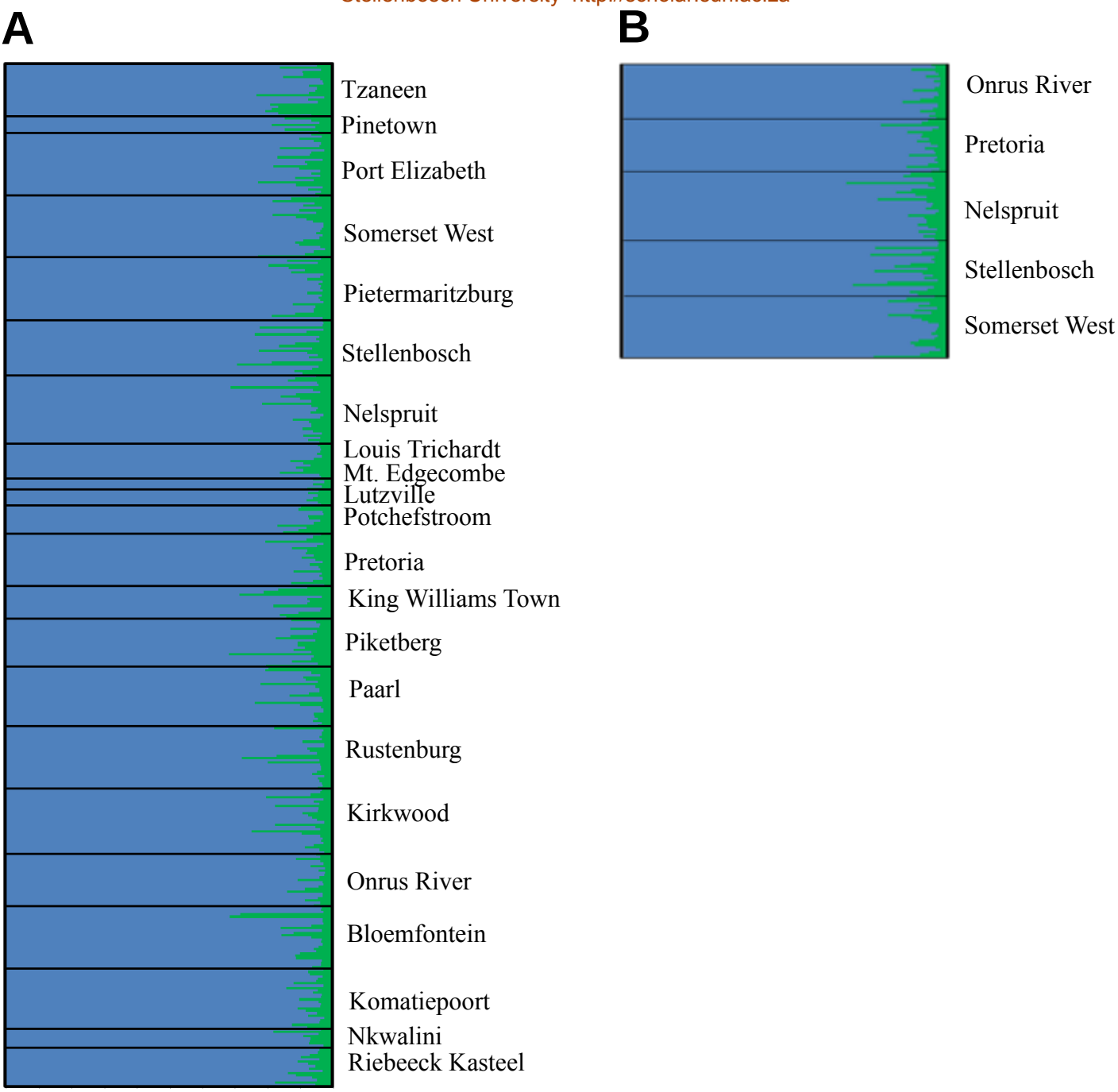


Figure 4.4 STRUCTURE results for (A) 458 *Ceratitis rosa* individuals from 22 South African populations for *K*=2 (B) a subset of locations for 129 *C. rosa* individuals from five sampling locations matching those of Virgilio *et al*. (2013).

Results from my AMOVA analysis indicated that variation was overwhelmingly partitioned within localities with 87.3% of variation accounted for by the within-population component (F_{ST}=0.127; p<0.0001; Table 4.3 (a)). Only 6.93% of pairwise F_{ST} comparisons between sites were significant (Table 4.4). Most of the significant differences were associated with the Kirkwood site, accounting for the significant F_{ST} results.

4.3.2 Morphometrics: wing shape

Results from the Procrustes ANOVA (Table 4.3 (b)) based on the error dataset showed that there was no significant measurement error (both digitizing and imaging error) (F=1.03, p = 0.302). Two hundred and sixty five individuals were included in the full dataset (Table 4.1) and a PCA for all individuals showed clear distinction between males and females (Fig. 4.5 (A), F=85.88, p<0.0001). In subsequent analyses I therefore separated the two sexes. For both males (F=4.15, p<0.0001) and females (F=5.96, p<0.0001) there was a significant side effect, indicative of directional asymmetry. The CVA plots for both males and females based on the first two canonical variables showed no clear discrimination between the 21 sampled locations (note, however, that in two locations I only obtained single-sex samples; in Potchefstroom there were no male individuals and in King Williams Town no females) (Fig. 4.5 (B); (C)). No pattern of IBD was detected when I plotted Mahalanobis distance against geographic distance for both males (r=0.014, p=0.834) and females (r=0.016, p=0.805) (Fig. 4.6 (A); (B)).

Table 4.3 Analysis of molecular variance (AMOVA) for *Ceratitis rosa* in South Africa (a) based on microsatellites calculated in ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010) and (b) a Procrustes ANOVA of shape variation for the error dataset based on a subset of my *C. rosa* wing shape measurements.

(a)

	d.f.	Sum of squares	Variance components	Percentage of variation	P
Among populations	21	268.155	0.269	12.72	<0.0001
Within populations	458	941.000	2.055	97.13	0.011

(b)

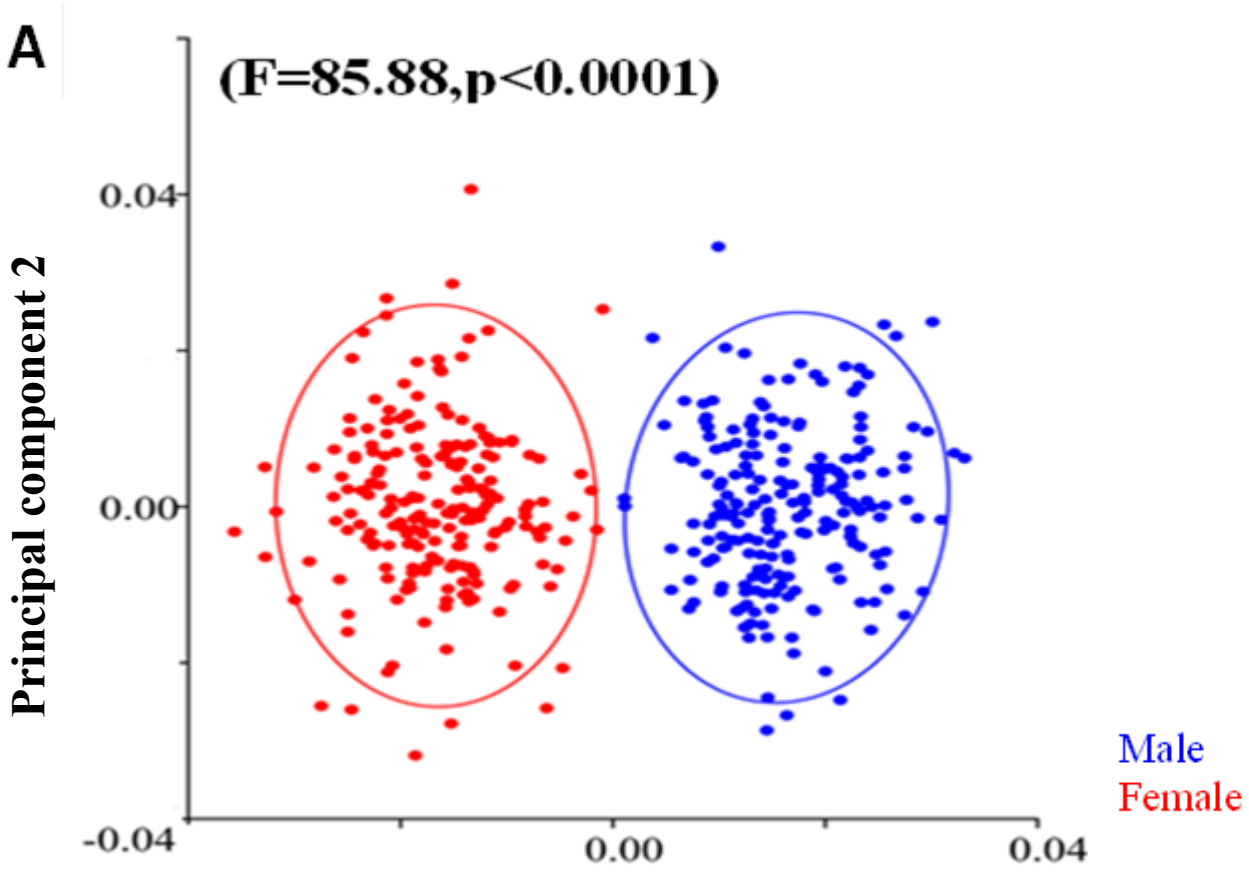
Effect	SS	MS	df	F	P(param)	Pillai tr.	P (param)
Individual	0.132	0.0002	576	11.81	<.0001	16.97	<.0001
Side	0.002	0.0001	24	3.23	<.0001	1	0.0813
Ind*Side	0.011	0.0000	576	19.82	<.0001	15.95	<.0001
Error 1	0.001	0.0000	864	1.03	0.302	7.06	0.5327
Residual	0.002	0.0000	2064				

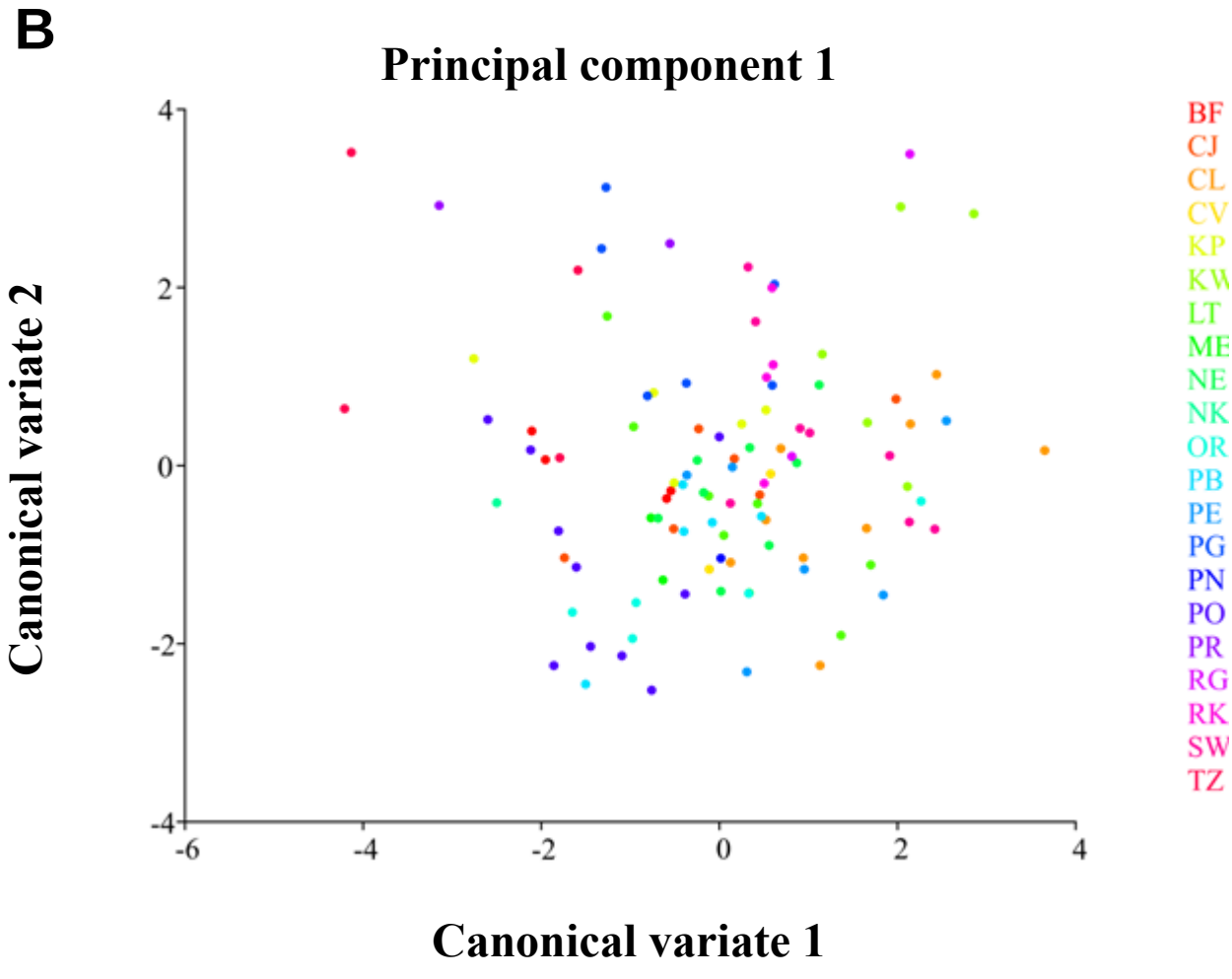
The table contains the Procrustes sums of squares (SS), Procrustes mean squares (MS), degrees of freedom (df), Goodall's *F* statistic (F) as well as the associated parametric *P*-value, and Pillai's trace with the associated parametric *P*-value.

Table 4.4 Pairwise F_{ST} values calculated from 11 polymorphic microsatellite markers for *Ceratitis rosa* sampled across South Africa. Names according to Table 4.1.

	BF	KT	KW	KP	LT	CV	ME	NE	NK	OR	CJ	PB	PG	PN	PE	PO	PR	RK	RG	SW	CL	TZ
BF	0.00																					
KT	0.00	0.00																				
KW	**0.03**	**0.05**	0.00																			
KP	0.01	0.00	**0.04**	0.00																		
LT	0.01	0.02	0.02	0.01	0.00																	
CV	0.02	0.01	0.05	0.04	0.02	0.00																
ME	0.00	0.00	0.01	0.00	0.00	0.00	0.00															
NE	0.01	0.02	0.02	0.01	0.00	0.03	0.00	0.00														
NK	0.02	0.01	0.05	0.01	0.02	0.03	0.00	0.03	0.00													
OR	0.02	0.02	**0.04**	0.02	0.00	0.02	0.00	0.01	0.01	0.00												
CJ	0.01	0.02	**0.03**	0.03	0.00	0.02	0.00	0.01	0.03	0.01	0.00											
PB	0.01	0.02	**0.04**	0.02	0.00	0.03	0.01	0.01	0.02	0.00	0.01	0.00										
PG	0.02	0.02	**0.05**	0.03	0.02	0.02	0.00	0.02	0.04	0.01	0.02	0.03	0.00									
PN	0.06	0.05	**0.08**	0.04	0.05	0.08	0.01	0.04	0.04	0.06	**0.09**	**0.07**	0.06	0.00								
PE	0.01	0.01	0.03	0.00	0.00	0.03	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.03	0.00							
PO	0.01	0.01	0.02	0.01	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.06	0.00	0.00						
PR	0.00	0.00	0.03	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.04	0.01	0.00	0.00					
RK	0.04	0.04	**0.06**	0.04	0.05	0.05	0.03	0.04	0.03	0.03	0.03	0.05	0.02	0.07	0.03	0.03	0.02	0.00				
RG	0.01	0.02	0.02	0.01	0.00	0.03	0.00	0.00	0.01	0.01	0.01	0.01	0.03	0.06	0.01	0.00	0.01	**0.05**	0.00			
SW	0.01	0.02	**0.04**	0.02	0.01	0.03	0.00	0.02	0.02	0.02	0.01	0.03	0.02	0.04	0.00	0.01	0.01	0.02	0.02	0.00		
CL	0.02	0.02	**0.05**	0.02	0.02	0.02	0.00	0.02	0.03	0.01	0.01	0.02	0.02	0.06	0.02	0.01	0.01	0.02	0.02	0.01	0.00	
TZ	0.02	0.01	**0.06**	0.01	0.02	0.04	0.00	0.02	0.01	0.03	0.03	0.03	0.03	0.02	0.01	0.02	0.01	**0.05**	0.02	0.02	0.03	0.00

Values in bold are significant ($p<0.05$).





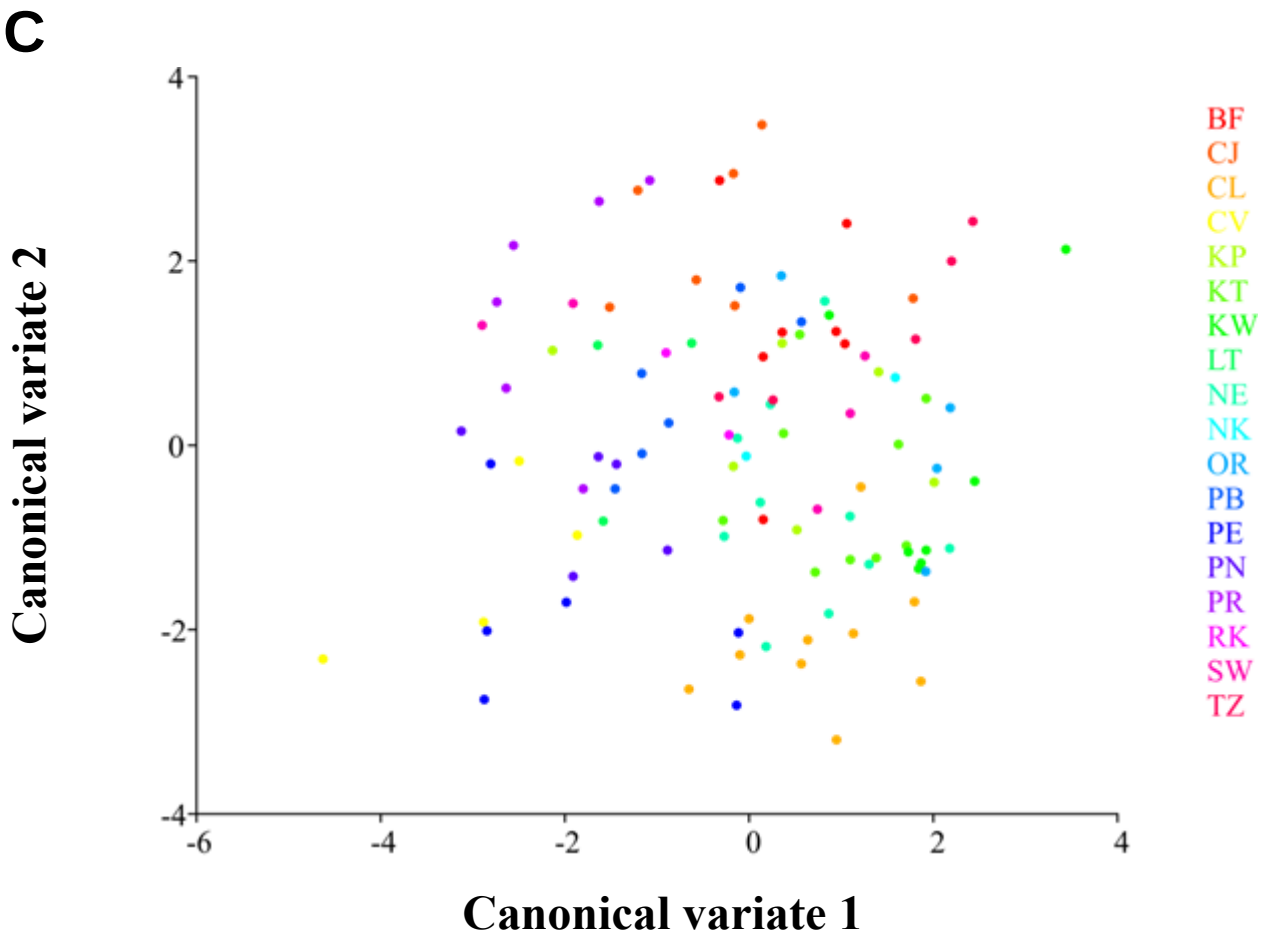


Figure 4.5 (A) Principal components analysis (PCA) of wing shape data for *Ceratitis rosa* individuals. Data points coloured according to sex. Ellipses represent 95% equal frequencies. Canonical Variate analysis of wing shape data for *Ceratitis rosa* individuals by location (ID as in Table 4.1) for (B) males and (C) females.

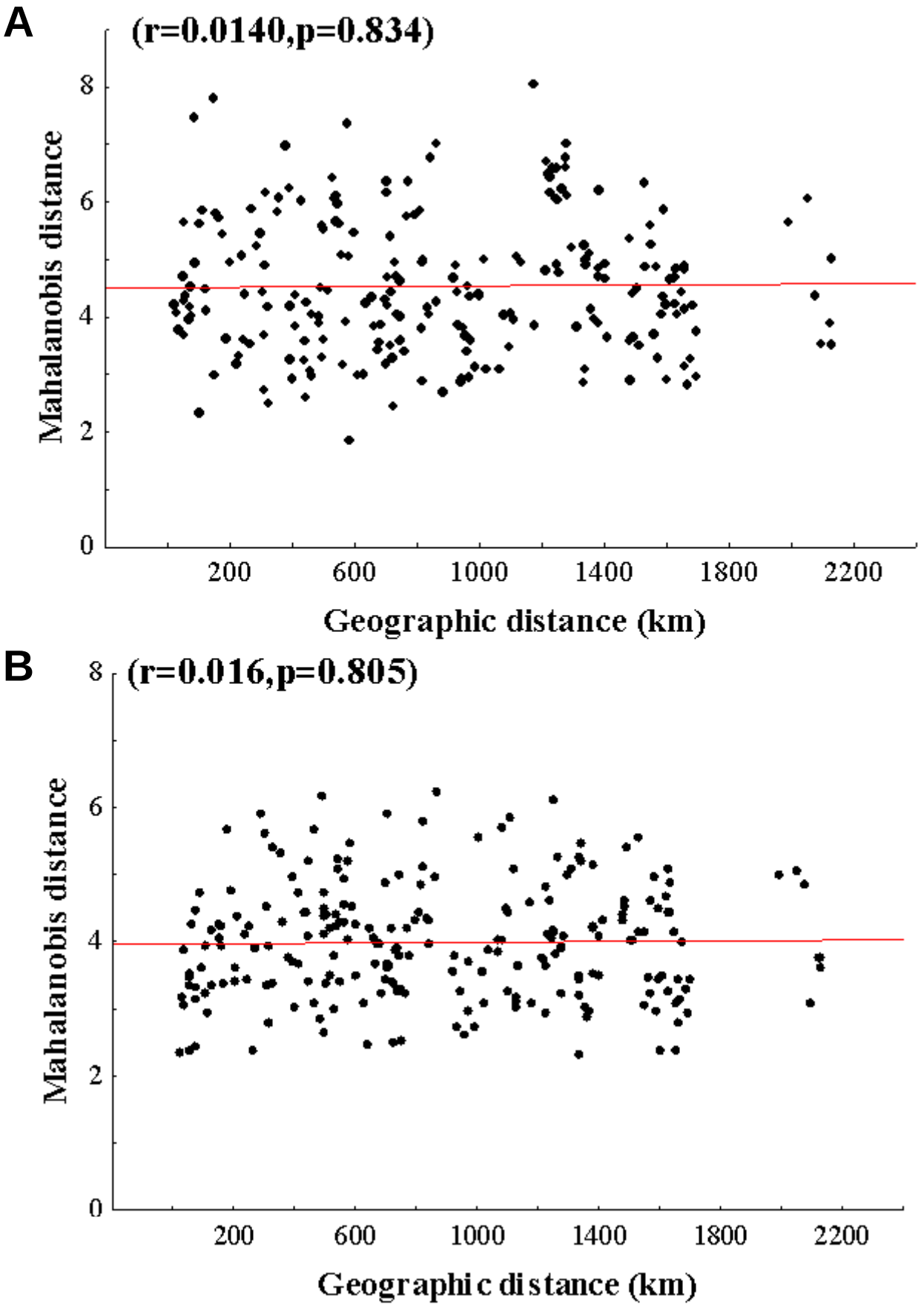


Figure 4.6 Linear correlation of Mahalanobis distance (from the CVA) against geographic distance between sampling locations (in km) (A) in males and (B) in females.

4.4 Discussion

The population genetics of pest species have been widely studied using molecular tools as well as Bayesian approaches. Yet surprisingly few studies to date have combined genetic data with phenotypic data (such as e.g. geometric morphometrics). Here I characterize genetic diversity and genetic structure alongside population discrimination based on geometric morphometrics. Results largely matched my hypothesis of one genotypic cluster (R2) over large areas of the country. Unexpectedly, however, I was unable to identify any individuals of the second morphotype (R1) at locations where it has previously been reported (see Virgilio *et al*. 2013). Furthermore, I found high levels of genetic diversity as well as rather unexpected low levels of interspecific population differentiation - between all sampling locations. In the complementary approach, geometric morphometric analyses showed that there were significant differences between males and females, but did not show inter-specific differentiation between different locations sampled either. These major findings are discussed in detail below.

Overall genetic diversity estimates for *C. rosa* in South Africa were high, but comparable to those of other studies for South Africa (H_E = 0.661, Baliraine *et al*. 2004; H_E= 0.682, Virgilio *et al*. 2013) and other mainland African populations, for example, Kenya (H_E= 0.70, Baliraine *et al*. 2004; H_E= 0.64, Virgilio *et al*. 2013) and Mozambique (H_E= 0.67, Virgilio *et al*. 2013). These estimates were similar to those of the Indian Ocean island, La Réunion, to which *C. rosa* spread within the last 60 years (De Meyer *et al*. 2008), based on the Baliraine *et al*. (2004) study (H_E=0.609), but higher than that of the Virgilio *et al*. (2013) study. These elevated levels of genetic diversity even on island populations indicates that, although *C. rosa* has not colonized many locations outside of its native range, when they are successfully established, populations have high effective population sizes. This may be at least partly due to their polyphagous nature and having a wide range of hosts available year-round to buffer populations against potential bottleneck events. Congruently, results from my Bottleneck analysis shows evidence of population expansion under both models investigated (TPM and SMM). This reservoir of high genetic diversity provides *C. rosa* with elevated levels of evolutionary potential, advantageous in the colonization process, which perhaps goes hand-in-hand with higher levels of phenotypic plasticity which can be considered an adaptive evolutionary advantage in the colonization of novel habitats (Lavergne & Molofsky 2007; Novak 2007; but see discussion in Nyamukondiwa *et al*. 2010 in the context of thermal tolerance plasticity in *Ceratitis*).

Based on results from my STRUCTURE analysis as well as inspection of morphological characters I found that there was only one morphotype (R2) present in all sampled locations in South Africa. Virgilio *et al*. (2013) included only six South African locations and showed that there was clear genetic structuring between the two *C. rosa* morphotypes (R1 and R2). Further, they showed that in two locations within South Africa both morphotypes occurred collectively (Nelspruit (Mpumalanga) and Marblehall (Limpopo) (based on GPS coordinates)) and the country seemed to lack pure populations of the R1 morphotype. Based on morphological inspection of the legs of *C. rosa* males, it is clear that both morphotypes (albeit R1 at low abundance) occur sympatrically in locations in the north of South Africa, especially in the Mpumalanga and Limpopo provinces (Tzaneen, Rustenburg, Komatiepoort, Nelspruit, Marblehall) (M. de Meyer, personal communication). I therefore expected to find only the one morphotype (R2) over large parts of South Africa based on the work by Virgilio *et al*. (2013) and post hoc inspection of the legs of male samples included here, yet flies sampled from the northern locations were also unexpectedly from only one morphotype. Explanations as to why I could only detect individuals of one type at locations where they were found previously are unknown. Possible explanations for this phenomenon can be the type of host plant in which development takes place as well the season in which individuals were sampled. When in time individuals are sampled goes hand in hand with temporal and climatic variability experienced in an individual's life-time. The lack of genetic differentiation between locations within the same morphotype (R2) suggests high levels of dispersal across large distances throughout South Africa which affects the implementation of successful area-wide pest management strategies. My data supports the implementation of a management plan at a much larger scale as the influence of humans on dispersal is evident over large distances. The need for improved area-wide pest management of fruit flies in South Africa has also been highlighted in previous studies assessing current management methods (Manrakhan & Addison 2014) and detailing the importance of alternate host pants for the spread of fruit flies (De Villiers *et al*. 2013a).

Although I initially set out to ascertain whether wing shape in *C. rosa* can be used to distinguish between the two morphotypes (R1 and R2), geometric morphometrics can also be used to investigate population structure. For example, Bouyer *et al*. (2007) showed significant shape differences between different populations of *Glossina palpalis gambiensis* sampled from Burkina Faso. The differences between the sampling localities based on wing shape were more prominent than the differences found from microsatellites. Based on my genetic results as well as morphological inspection of *C. rosa* individuals, South Africa appears to

mainly have only one type (R2), at the time I sampled them, with no intraspecific differentiation between sampling localities. Results from the geometric morphometric analyses supported these findings. I showed that *C. rosa* is sexually dimorphic based on wing shape. Sexual dimorphism has mainly been investigated as differences in body size (Fairbairn *et al*. 2007). However, in nine species in the *Drosophila melanogaster* subgroup sexual dimorphism based on wing shape was investigated and all nine species exhibited sexual dimorphism (Gidaszewski *et al*. 2009). Sexual dimorphism in tephritids may be linked to courtship behaviour as males use many different types of stimuli including wing movements to attract the attention of females (Wicker-Thomas 2007). After separation of males and females I found no differentiation between sampling localities based on wing shape. Still, information from wing shape as a discriminator between species is vital to support molecular studies.

4.4.1 Conclusions and future directions

Here I showed that South African populations of *C. rosa* are largely of only one type (R2) without any differentiation between different locations sampled based on both microsatellite data and geometric morphometric data. Future research is required to determine whether the two morphotypes (R1 and R2) are different based on ecological requirements as well as physiology. Work should focus on their environmental niches and changes in the abundance of morphotypes in an area between different seasons. Moreover, the possibility of these traits being sex-linked (Nylin *et al*. 1994; Ellers & Boggs 2002; Qvarnström & Bailey 2008) or under frequency-dependent selection (Mallet & Joron 1999; Borer *et al*. 2010) should be investigated. Furthermore, results suggest that there are high levels of gene flow throughout South Africa with the influence of humans being implicated. Although these flies can move considerable distances through natural dispersal, aided by the distribution of suitable host plants, the possibility that many of these events have been human-mediated are likely. These high levels of dispersal play an important role in the successful implementation of an integrated pest management program and suggest that these programs need to take into account the distribution of a species both spatialy and temporaly. Therefore based on results from this study control measures need to be implemented on larger spatial scales (country-wide), however these recommendations are conceivably impractical, especially in South Africa. Eradicating *C. rosa* from South Africa completely is improbable and we should therefore rather aim for suppression of this species. The integration of different types of control (chemical application and biological control) as well as cooperation between landowners is crucial as the most important driver of dispersal patterns seen here is closely

linked to the movement of agricultural products within the country limits. In Hawaii for example they implemented an education and outreach component to their area-wide fruit fly management program which targeted all growers (commercial and backyard) as well as communities (Mau *et al*. 2007). Moreover, in Australia they have a fruit fly exclusion zone in which total control are maintained together with community awareness programs highlighting the negative impacts of these pests (Jessup *et al*. 2007). Similar programs in South Africa may assist in the eradication or atleast suppression of these flies in some fruit-growing areas in South Africa.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

The invasion of invertebrate pests is likely to increase in future, closely linked not only to increased trade of goods worldwide but also climate change (Kirk *et al*. 2013). These new invasions together with the increase in human population growth will place rising pressure on agricultural productivity (Kirk *et al*. 2013). Proposed fields of research need to encompass the different stages of the invasion process (Blackburn *et al*. 2011) including prevention and control/mitigation of biological invasions. The Tephritidae constitute many successful invaders (e.g. *Bactrocera dorsalis* and *Ceratitis capitata*) and different fields of research have applications to further understand the invasion process.

The main aims of this dissertation were to:

1. investigate seasonal variation in fruit fly abundance in orchards and natural vegetation in the Western Cape to determine whether natural vegetation is used as possible refugia;
2. investigate global population structure of *C. capitata* with a focus on southern Africa to reconstruct and test *C. capitata's* invasion pathway using a Bayesian framework;
3. investigate the population genetic structure, using molecular and morphological markers, to estimate gene flow and dispersal ability of *C. rosa* in South Africa.

Here I briefly discuss thr role the use of genetics play, I highlight the main results from the different research chapters and discuss some important implications of my research. Moreover, I provide my thoughts on future research avenues and make concluding remarks.

Many factors including the mode of dispersal, the landscape organization as well as the number of introductions all influences the spread of an invasive species. Genetics have been shown to be valuable in addressing some of these questions important in managing and preventing new invasions. These include for example: the identification of pest species and different mechanisms of species resistance to pesticides as well as assessing whether pest management practises are effective. Genetics are also useful to reconstruct invasion pathways or routes of colonisation as well as other demographic processes such as models of dispersal and population bottlenecks (Fitzpatrick *et al*. 2012; Kirk *et al*. 2013).

The first way in which genetics can help prevent invasions is by aiding the correct idenitifcation of a new invasive species. Although most fruit flies are readily identifiable using morphological keys when they are mature, immature stages however prove difficult to

identify, especially in the field (White & Elson-Harris 1994). This is also true for fruit flies that form part of species complexes that are notoriously hard to identify based on morphology alone. Molecular tools have proven to be invaluable to identify immature stages to species level (Douglas & Haymer 2001; Baliraine *et al*. 2003) as well as those species part of species complexes (Schutze *et al*. 2012a; Virgilio *et al*. 2013). A recent example is that of *B. invadens* in Africa which after much debate has been placed in the *Bactrocera dorsalis* complex and is not a separate entity as previously thought.

The second way in which genetics can assist in understanding new invasions is determining the native range of a species. The determination of the native range can help in identifying biological control agents to aid management of introduced species (Kirk *et al*. 2013).

The third way in which genetics can help prevent new invasions or managing exsisting ones is to reconstruct invasion pathways, identify migrants or whether species were introduced by a single introduction or multiple introductions. This goes hand in hand with understanding whether new fly interceptions are new introductions or outbreaks or in actual fact just a range expansion of an already established population (Malacrida *et al*. 2007). Knowing where the new introductions are coming from can aid countries in targeting their efforts on particular countries, trade routes or ports to prevent further introduction.

Understanding the dynamics of an invasive (or resident) fruit fly in a country can also be useful in managing the pest. This entails investigating the dispersal ability of a species using molecular tools. If for example there are structured population in a region it is clear that no movement is taking place between these locations and management can focussing on implementing area-wide pest management strategies on the different groups (management units), employing eradication programs in specific regions or preventing spread between these identified units. If however, a lack of differentiation is found management of that area becomes much harder and suppression of population numbers are more likely than eradication. These dispersal distances can be used to identify quarantine borders in areas under fruit fly control.

Finally, some consideration needs to be given to species in which different genotypes or clusters are identified with regards to the effectiveness of biological control agents as well as other biochemical and physiological traits of the individuals from the different clusters that can influence management practices.

All the above mentioned examples of benefits of genetics to invasion management also come with some limitations. The first limitation is the limitation on statistical power of models used to reconstruct invasion pathways (Estoup & Guillemaud 2010). The most obvious constraint is incomplete sampling of a species range. The second limitation is the number of molecular markers employed in a study (Kirk & Freeland 2011) and finally other explanations of observed genetic structure patterns and the interpretation of these patterns (Kirk *et al*. 2013). Despite these limitations however molecular tools in resolving some important issues and can serve to compliment other approaches.

In Chapter 2, I found that despite using multiple lures (Biolure®, Cue Lure, Olive Fly Lure and Questlure) *Ceratitis capitata* and *C. rosa* were the only species detected at high numbers. Biolure® was the most effective lure with the highest trap catches for both *C. capitata* and *C. rosa* in austral autumn (March-May) in both orchards and natural vegetation. I also showed that there was a significant difference between natural vs. orchard habitat types, with orchards typically containing greater numbers of both species. No evidence was found to suggest that flies are moving between natural vegetation and orchards during periods of limited host availability.

The detection of new invasions as well as simultaneously monitoring the economic population thresholds for the control of fruit flies depends heavily on the efficacy of lures. This then poses the question whether these species are really not present or whether they are simply persisting at low numbers. Papadopoulos *et al*. (2013) argues that even when fruit fly populations persist at undetectable levels, they may in actual fact have already established and therefore nullifies the concept of "early detection". This statement has led to a heated debate regarding when a species is considered established and what approach is more favourable to use in predicting areas of establishment (Gutierrez *et al*. 2014; Carey *et al*. 2014). Howerev, many fruit fly species (especially the less abundant species) are not found in managed orchards and to detect these will require the sampling of additional habitat types as well as implementing various sampling methods.

In Chapter 3, I showed that for *C. capitata* there are high levels of genetic diversity on the African continent with a decline in genetic diversity in the introduced range (Australia, Greece, Guatemala and Madeira). I also found that there is a clear pattern of population structure between Africa and all other locations sampled in the introduced range (Australia,

Greece, Guatemala and Madeira). Burgers Hall (South Africa) was the only exception and grouped more closely with those locations in the introduced range (Australia, Greece, Guatemala and Madeira) possibly indicating recent migration of *C. capitata* individuals into this population from outside of the native range. Based on this information I conclude that South Africa indeed forms part of the native range of *C. capitata*. After reconstructing and testing three *a priori* hypotheses of invasion pathways for *C. capitata* using Bayesian inference I found that the statistically supported scenario closely matched the dates of colonization available. This scenario comprised the colonization of Europe from Africa (native range), a secondary colonization of Australia from Europe (admixture between Greece and Madeira) and finally the colonization of the Americas from Africa.

Information from the reconstruction of invasion pathways is important in understanding a multitude of factors that make for successful invasions. This information can be incorporated into strategies for control and prevention of new invasions (Estoup & Guillemaud 2010). For *C. capitata*, I show that connectivity between the African continent and the introduced range is low. This indicates that quarantine measures, including high levels of control, as well as post-harvest treatments, are likely successful in removing flies from export consignments or preventing the ongoing spread of this species through commercial trade routes internationally. However, on the African continent there is evidence for high levels of movement. Given the distances covered and the likely associated speed of movement, this probably constitutes human-assisted movement. Consequently, efforts for quarantine measures between different countries on the African continent require refining. These include collaboration between government agricultural agencies in different countries, public education on the importance of fruit fly control and the improvement of border control and policing. A case in point is *Bactrocera invadens* Drew, Tsuruta & White which was recently detected (2010) in South Africa for the first time (Manrakhan *et al*. 2011) after its introduction to the African continent in 2003 (Lux *et al*. 2003). Despite extensive efforts, after its introduction it has spread rapidly over large parts of the African continent (De Meyer *et al*. 2010). I reconstructed possible routes of invasion based on limited sampling of the extensive distribution of *C. capitata* (Chapter 3). Information gained is however limited as many more sampling locations are required to enhance the predictive and explanatory power of analyses presented.

In Chapter 4, using information from microsatellites and geometric morphometrics, I showed that *C. rosa* individuals from South Africa are largely from only one morphological type (R2) despite including locations where individuals from both types (R1 and R2) were recovered previously (see Virgilio *et al*. 2013). Moreover, within the R2 morphotype I recovered high levels of genetic diversity and little to no population differentiation across all populations sampled. Congruent to the results from microsatellite genetic markers, I also show the absence of interspecific differentiation between sampling locations based on wing shape. This indicates high levels of movement between different sites. This movement is likely characterised by natural dispersal (i.e. flight over short and long distances) and human-mediated dispersal through trade and human travel, however, disentangling natural dispersal and human-assisted dispersal is difficult at this time.

As in *C. capitata* (Karsten *et al*. 2013), *C. rosa* showed high levels of gene flow throughout South Africa. Although flies can travel substantial distances by natural dispersal (i.e. flight), movement between distant sampling localities are possibly also linked to human-mediated dispersal. Moreover, these high levels of dispersal have profound effects on integrated pest management as it is currently implemented. A management unit should therefore comprise multi-owner fruit orchards over provincial lines as well as home gardens and wild host areas over a much larger scale than currently implemented.

An additional limitation on the use of molecular tools to determine indirect measures of dispersal is the repeated disagreement between direct measurements and estimates of gene flow. Some studies have shown that estimates of gene flow can be directly compared to direct estimates of dispersal (Bohonak 1999; Peterson & Denno 1998). For example in *C. caipitata* the direct estimates of dispersal using mark-recapture measurements (Meats & Smallridge 2007) are significantly smaller than those from molecular estimates (Karsten *et al*. 2013). One of the most important considerations (and criticisms) is the temporal scales over which the estimation of dispersal is measured using genetic tools (Berry *et al*. 2004; Vandewoestijne and Baguette, 2004*).* When using population structure and assignment tests to infer dispersel we need to keep this limitation in mind as our population structure hypotheses are ofthen over-simplified, our markers are sensitive to temporal scales and there are also other factors that influence our measurements including selection and genetic drift.

5.1 Future directions

In the light of monitoring fruit flies for control, effective lures, are required. Intercepting new invasions as well as host switches are important in fruit fly management which goes hand in hand with correctly identifying the species. Failure to do so can have disastrous consequences, for example, the incorrect identification of *Drosophila suzukii* in the USA lead to the pest spreading large distances undeterred and causing considerable economic damage to agricultural production (Hauser 2011). The development of effective lures that is highly attractive to a range of species and work over large distances is therefore crucial. Results from Chapter 2 can be strengthened by monitoring sites over a longer period of time, quantifying the availability of resources in the different habitat types as well as the measurement of various abiotic variables (temperature, relative humidity and rainfall) in the different habitat types. Actively searching for additional fruit fly species in the natural vegetation should be done by collecting fruit for rearing from natural habitat as well as employing other types of sampling for example netting. To further assess the efficacy of the lures tested in this chapter the response of different fruit flies will need to be tested under controlled conditions, either in cages or in the laboratory.

To more accurately assess and confirm the proposed route of the *C. capitata* invasion worldwide samples from many more countries need to be included. This will allow for definite conclusions made on the route of invasion from Africa to the world a well as secondary colonization events.

Understanding the temporal dynamics of fruit flies is critical to further the development of integrated control strategies and sustainable agriculture in South Africa. Based on microsatellite genetic markers investigated in this dissertation (Chapter 4) I did not recover the two morphotypes (R1 and R2) previously identified within South Africa (Virgilio *et al*. 2013). The absence of one of the morphotypes at sampling localities where they were previously recovered begs the question of whether the two morphotypes have different environmental niches, whether their abundance change seasonally or if the associated traits are sex-linked or under frequency-dependent selection? Future work should therefore investigate the temporal variability of the two *C. rosa* morphotypes this will help to elucidate whether the same suit of control methods can be used to target the two morphotypes, as biological control methods are often highly species-specific. A possible experiment to answer some of these questions is establishing a pure colony of one type (R1 or R2) and dividing the

colony into different treatment groups. Each group will undergo various treatments to for example induce seasonal changes under different climate types (temperate *versus* sub tropical), rearing on different hosts or a combination of both.

With the proposed dispersal distances of *C. rosa* in South Africa alternative methods for control need to be investigated. The control of fruit flies in South Africa are predominantly achieved by chemical applications but due to their potential negative environmental impacts as well as concerns on insecticide resistance (Vreysen *et al*. 2007) more environmentally-friendly methods are preferable. Little is known about other potential biological control methods for *Ceratitis* spp. in South Africa. Some of the alternatives of chemical control are microbial control agents (MCAs), including entomopathogenic- fungi (EPFs) and nematodes (EPNs). Therefore the identification and assessment of alternative biological control agents specifically in the form of fungi and nematodes are of value due to their target specificity, environmental safety and incorporation with other methods, such as the Sterile Insect Technique (SIT). The use of sterile males to disseminate fungal conidia integrated into a SIT program has been explored in Mexico (Toledo *et al*. 2006). In South Africa, Malan & Manrakhan (2009) showed that entomopathogenic nematodes may be a viable option for the biological control of *C. capitata* and *C. rosa*. Moreover, in laboratory trials entomopathogenic fungal isolates have shown some potential (Goble *et al*. 2011). In Kenya, *Beauveria bassiana* and *Metarhyzium* spp., has demonstrated potential as they have high rates of infection against *Ceratitis* spp. (Ekesi *et al*. 2002; Dimbi *et al*. 2009). Information gained on biological control of *Ceratitis* spp. can be incorporated into integrated management programs for fruit flies in South Africa on the road to reducing the chemical inputs required for successful control.

5.2 Concluding remarks

In this dissertation I aimed to attain knowledge on basic and applied biology of fruit flies that may feed into management plans to prevent new invasions and aid the development of effective integrated area-wide pest management control strategies. Balancing food production through sustainable agriculture with economic, social and political pressures is challenging. Despite the limits of available knowledge there are some consistent themes emerging. These include the influence of humans on new invasions and pest mitigation, the interplay between species and different locations, dispersal distances hampering area-wide integrated pest management programs and propagule pressure. Our understanding of integrated pest

management (and new invasions) will be greatly enhanced by addressing some important gaps. First, we need to further investigate the role of non-crop hosts for economic important fruit flies in South Africa and their influence on population dynamics in an area. Second, for morphospecies or species that contain multiple morphotypes (genetic clusters) understanding biochemical and physiological processes will be important to assess the efficacy of post-harvest methods based on gas and temperature treatments. The most important question to answer will be whether these genetic differences translate into phenotypic differences important for varied fitness and survival between them. Third, the use of natural enemies (EPFs and EPNs) in integrated programs for the control of fruit flies need to be explored as not much is known for fruit flies in South Africa. Finally, due to the disparity between indirect and direct measures of dispersal of fruit flies resolving the true flight distance of a species is of significance. Designing a way to accurately measure dispersal distance will be valuable and will impact quarantine distances as well as SIT.

REFERENCES

Aketarawong N, Bonizzoni M, Thanaphum S, Gomulski LM, Gasperi G, Malacrida AR, Gugliemino CR (2007) Inferences on the population structure and colonization process of the invasive oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Molecular Ecology* **16**: 3522-3532.

Aketarawong N, Guglielmino CR, Karam N, Falchetto M, Manni M, Scolari F, Gomulski LM, Gasperi G, Malacrida AR (2014) The oriental fruit fly *Bactrocera dorsalis s.s.* in East Asia: disentangling the different forces promoting the invasion and shaping the genetic make-up of populations. *Genetica* **142**: 201-213.

Alaoui A, Imoulan A, Alaoui-Talibi Z El, Meziane A El (2010) Genetic structure of Mediterranean fruit fly (*Ceratitis capitata*) populations from Moroccan Endemic Forest of *Argania spinosa*. *International Journal of Agriculture & Biology* **12**: 291-298.

Aluja M, Celedonio-Hurtado H, Liedo P, Cabrera M, Castillo F, Guillén J, Rios E (1996) Seasonal population fluctuations for management of *Anastrepha* fruit flies (Diptera: Tephritidae) in commercial mango orchards in Southern Mexico. *Journal of Economic Entomology* **89**: 654-667.

Annecke DP, Moran VC (1982) Insects and Mites of Cultivated Plants in South Africa. Butterworths, Durban.

Archer D, Rahmstorf S (2010) The climate crisis: An introductory guide to climate change. Cambridge University Press, Cambridge.

Augustinos AA, Stratikopoulos EE, Zacharopoulou A, Mathiopoulos KD (2002) Polymorphic microsatellite markers in the olive fly, Bactrocera oleae. *Molecular Ecology Notes* **2**: 278-280.

Augustinos AA, Mamuris Z, Stratikopoulos EE, D'Amelio S, Zacharopoulou A, Mathiopoulos KD (2005) Microsatellite analysis of olive fly populations in the Mediterranean indicates a westward expansion of the species. *Genetica* **125**: 231-241.

Baliraine FN, Bonizzoni M, Osir EO, Lux SA, Mulaa FJ, Zheng L, Gomulski LM, Gasperi G, Malacrida AR (2003) Comparative analysis of microsatellite loci in four fruit fly

species of the genus *Ceratitis* (Diptera: Tephritidae). *Bulletin of Entomological Research* **93**: 1-10.

Baliraine FN, Bonizzoni M, Guglielmino CR, Osir EO, Lux SA, Mulaa FJ, Gomulski LM, Zheng L, Quilici S, Gasperi G, Malacrida AR (2004) Population genetics of the potentially invasive African fruit fly species, *Ceratitis rosa* and *Ceratitis fasciventris* (Diptera: Tephritidae). *Molecular Ecology* **13**: 683-695.

Barclay HJ, Matlock R, Gilchrist S, Suckling DM, Reyes J, Enkerlin WR, Vreysen MJB (2011) A conceptual model for assessing the minimum size area for an area-wide integrated pest management program. *International Journal of Agronomy*, available online at doi.org/10.1155/2011/409328.

Barnes BN (2000) Fruit flies of economic importance in South Africa: an overview. In: Fruit Fly Symposium Proceedings, pp. 101-107.

Barnes BN, Eyles DK (2000) Feasibility of eradicating *Ceratitis* spp. fruit flies from the Western Cape of South Africa by the sterile insect technique. In: Area-wide control of fruit flies and other insect pests, pp. 449-455.

Barnes BN, Eyles DK, Franz G (2002) South Africa's fruit fly SIT programme- the Hex River Valley pilot project and beyond. In: 6th International Fruit Fly Symposium, pp. 131-141.

Barnes BN, Venter J-H (2006) The South African fruit fly action plan- Area-wide suppression and exotic species surveillance. In: Proceedings of the 7th International Symposium of Fruit flies of Economic Importance, pp. 271-283.

Barr NB, McPheron BA (2006) Molecular phylogenetics of the genus *Ceratitis* (Diptera: Tephritidae). *Molecular Phylogenetics & Evolution* **38**: 216-230.

Barr NB, Copeland RS, De Meyer M, Masiga D, Kibogo HG, Billah MK, Osir E, Wharton RA, McPheron BA (2006) Molecular diagnostics of economically important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and RFLP analyses. *Bulletin of Entomological Research* **96**: 505-521.

Barr NB (2009) Pathway Analysis of *Ceratitis capitata* (Diptera: Tephritidae) Using Mitochondrial DNA. *Journal of Economic Entomology* **102**: 401-411.

Barrett SC (1992) Genetics of weed invasions. In *Applied population biology*. Springer Netherlands.

Baruffi L, Damiani G, Guglielmino CR, Bandi C, Malacrida AR, Gasperi G (1995) Polymorphism within and between populations of *Ceratitis capitata* - Comparison between RAPD and Multilocus Enzyme Electrophoresis Data. *Heredity* **74**: 425-437.

Bebber DP, Ramotowski MA, Gurr SJ (2013) Crop pests and pathogens move polewards in a warming world. *Nature Climate Change* **3**: 985-988.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996-2004) GENETIX 4.05, *logiciel sous Windows TM pour la génétique des populations*. Montpellier: Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal?. *Molecular Ecology* **13**: 551-561.

Berthouly-Salazar C, Hui C, Blackburn TM, Gaboriaud C, Van Rensburg BJ, Jansen van Vuuren B, Le Roux JJ (2013) Long-distance dispersal maximizes evolutionary potential during rapid geographic range expansion. *Molecular Ecology* **22**: 5793-5804.

Bess HA, van den Bosch R, Haramoto FH (1961) Fruit fly parasites and their activities in Hawaii. *Proceedings of the Hawaiian Entomological Society* **27**: 367–378.

Bezzi M (1913) Indian Tephritids (fruit flies) in the collection of the Indian Museum, Calcutta. *Memoirs of the Indian Museum* **3:** 153–175.

Bitner-Mathé BC, Klaczko LB (1999) Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata* wings. *Heredity* **83**: 688-696.

Blackburn TM, Lockwood JL, Cassey P (2009) Avian Invasions-The ecology and evolution of exotic birds. Oxford University Press, New York.

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* **26**: 333-339.

Blackburn TM, Prowse TA, Lockwood JL, Cassey P (2013) Propagule pressure as a driver of establishment success in deliberately introduced exotic species: fact or artefact? *Biological Invasions* **15**: 1459-1469.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. *Quarterly Review of Biology* **74**: 21-45.

Bomfim ZV, Lima KM, Silva JG, Costa MA, Zucchi RA (2011) A morphometric and molecular study of *Anastrepha pickeli* Lima (Diptera: Tephritidae). *Neotropical Entomology* **40**: 587-594.

Bonizzoni M, Malacrida AR, Guglielmino CR, Gomulski LM, Gasperi G, Zheng L (2000) Microsatellite polymorphism in the Mediterranean fruit fly, *Ceratitis capitata*. *Insect Molecular Biology* **9:** 251-261.

Bonizzoni M, Zheng L, Guglielmino CR, Haymer DS, Gasperi G, Gomulski LM, Malacrida AR (2001) Microsatellite analysis of medfly bioinfestations in California. *Molecular Ecology* **10**: 2515-2524.

Bonizzoni M, Katsoyannos BI, Marguerie R, Guglielmino CR, Gasperi G, Malacrida A, Chapman T (2002) Microsatellite analysis reveals remating by wild Mediterranean fruit fly females, *Ceratitis capitata*. *Molecular Ecology* **11**: 1915-1921.

Bonizzoni M, Guglielmino CR, Smallridge CJ, Gomulski M, Malacrida AR, Gasperi G (2004) On the origins of medfly invasion and expansion in Australia. *Molecular Ecology* **13**: 3845-3855.

Bookstein FL (1991) Morphometric tools for landmark data: Geometry and biology. Cambridge University Press, New York.

Borer M, Van Noort T, Rahier M, Naisbit RE (2010) Positive frequency-dependent selection on warning color in alpine leaf beetles. *Evolution* **64**: 3629-3633.

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* **144**: 1-11.

Boykin LM, Shatters RG, Hall DG, Burns RE, Franqui RA (2006) Analysis of host preference and geographical distribution of *Anastrepha suspensa* (Diptera:

Tephritidae) using phylogenetic analyses of mitochondrial cytochrome oxidase I DNA sequence data. *Bulletin of Entomological Research* **96**: 457-469.

Boykin LM, Shatters RG, Hall DG, Dean D, Beerli P (2010) Genetic variation of *Anastrepha suspensa* (Diptera: Tephritidae) in Florida and the Caribbean using microsatellite DNA markers. *Journal of Economic Entomology* **103**: 2214-2222.

Bouyer J, Ravel S, Dujardin JP, De Meeüs T, Vial L, Thévenon S, Guerrini L, Sidibé I, Solano P (2007) Population structuring of *Glossina palpalis gambiensis* (Diptera: Glossinidae) according to landscape fragmentation in the Mouhoun river, Burkina Faso. *Journal of Medical Entomology* **44**: 788-795.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* **368**: 455-457.

Broquet T, Petit EJ (2009) Molecular estimation of dispersal for ecology and population genetics. *Annual Review of Ecology, Evolution & Systematics* **40**: 193-216.

Broughton S, De Lima CPF (2002) Field evaluation of female attractants for monitoring *Ceratitis capitata* (Diptera: Tephritidae) under a range of climatic conditions and population levels in Western Australia. *Journal of Economic Entomology* **95**: 507-512.

Caccone A (1985) Gene flow in cave organisms: A qualitative and quantitative approach. *Evolution* **39**: 1223-1235.

Caccone A, Sbordoni V (1987) Molecular evolutionary divergence among North American cave crickets. I. Allozyme variation. *Evolution* **41**: 1198-1214.

Cantrell B, Chadwick B, Cahill A (2002) Fruit Fly Fighters – Eradication of the Papaya Fruit Fly. 200 pp. Collingwood, Australia, Csiro.

Carey JR, Plant RE, Papadopoulos NT (2014) Response to commentary by Gutierrez *et al*. *Proceedings of the Royal Society B* **281**, available online at doi: 10.1098/rspb.2013.2989.

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology & Evolution* **24**: 621-631.

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes* **7**: 747-756.

Clarke AR, Armstrong KF, Carmichael AE, Milne JR, Raghu S, Roderick GK, Yeates DK (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology* **50**: 293-319.

Clarke AR, Powell KS, Weldon CW, Taylor PW (2011) The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management?. *Annals of Applied Biology* **158**: 26-54.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics* **20**: 2363-2369.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001-2014.

Cornuet JM, Santos F, Beaumont MA, Robert CP, Marin J-M, Balding DJ, Guillemaud T, Estoup A. (2008) Inferring population history with DIY ABC, a user-friendly approach to approximate Bayesian computation. *Bioinformatics* **24**: 2713-2719.

Cornuet JM, Ravigné V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics* **11**: 401-411.

Cornuet JM, Pudlo P, Veyssier J, Dehne-Garcia A, Gautier M, Leblois R, Marin J-M, Estoup A (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* **30**: 1187-1189.

Delatte H, Virgilio M, Simiand C, Quilici S, De Meyer M (2013) Isolation and characterisation of sixteen microsatellite markers cross-amplifying in a complex of three African agricultural pests (*Ceratitis rosa*, *C. anonae* and *C. fasciventris*, Diptera: Tephritidae). *Conservation Genetics Resources* **5**: 31-34.

De Meyer M (2000) Systematic revision of the subgenus *Ceratitis* MacLeay *s.s.* (Diptera, Tephritidae). *Zoological Journal of the Linnean Society* **128**: 439-467.

De Meyer M (2001) On the identity of the Natal fruit fly *Ceratitis rosa* Karsch (Diptera, Tephritidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **71**: 55-62.

De Meyer M, Copeland RS, Wharton RA, McPheron BA (2002) On the geographic origin of the Medfly *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae). In: Proceedings of the 6th International Fruit Fly Symposium, pp. 45-53.

De Meyer M (2005) Phylogenetic relationships within the fruit fly genus *Ceratitis* MacLeay (Diptera: Tephritidae), derived from morphological and host plant evidence. *Insect Systematics & Evolution* **36**: 459-479.

De Meyer M, Robertson MP, Peterson AT, Mansell MW (2008) Ecological niches and potential geographical distributions of Mediterranean fruit fly (*Ceratitis capitata*) and Natal fruit fly (*Ceratitis rosa*). *Journal of Biogeography* **35**: 270-281.

De Meyer M, Robertson MP, Mansell MW, Ekesi S, Tsuruta K, Mwaiko W, Vayssières J-F, Peterson AT (2010) Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). *Bulletin of Entomological Research* **100**: 35-48.

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society* **39**: 1-38.

Dent D (2000) *Insect pest management*. Cabi.

De Villiers M, Manrakhan A, Addison P, Hattingh V (2013a) The distribution, relative abundance, and seasonal phenology of *Ceratitis capitata*, *Ceratitis rosa*, and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. *Environmental Entomology* **42**: 831-840.

De Villiers M, Hattingh V, Kriticos DJ (2013b) Combining field phenological observations with distribution data to model the potential distribution of the fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin of Entomological Research* **103**: 60-73.

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**: 167-169.

Dimbi S, Maniania NK, Ekesi S (2009) Effect of *Metarhizium anisopliae* inoculation on the mating behavior of three species of African Tephritid fruit flies, *Ceratitis capitata, Ceratitis cosyra* and *Ceratitis fasciventris*. *Biological Control* **50**: 111-116.

Dixon AF, Honěk A, Keil P, Kotela MAA, Šizling AL, Jarošík V (2009) Relationship between the minimum and maximum temperature thresholds for development in insects. *Functional Ecology* **23**: 257-264.

Douglas LJ, Haymer DS (2001) Ribosomal ITS1 polymorphisms in *Ceratitis capitata* and *Ceratitis rosa* (Diptera: Tephritidae). *Annals of the Entomological Society of America* **94**: 726-731.

Drew RA, Hancock DL (1994) The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research Supplement Series* **2**: 1-68.

Drew RAI, Tsuruta K, White IM (2005) A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology* **13**: 149-154.

Drotz MK (2003) Speciation and mitochondrial DNA diversification of the diving beetles *Agabus bipustulatus* and *A. wollastoni* (Coleoptera, Dytiscidae) within Macaronesia. *Biological Journal of the Linnean Society* **79**: 653-666.

Dryden IL, Mardia KV (1998) Statistical shape analysis (Vol. 4). John Wiley & Sons, New York.

Dujardin JP, Pont FL, Baylac M (2003) Geographical versus interspecific differentiation of sand flies (Diptera: Psychodidae): a landmark data analysis. *Bulletin of Entomological Research* **93**: 87-90.

Durand E, Jay F, Gaggiotti OE, François O (2009) Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology & Evolution* **26**: 1963-1973.

Duyck PF, Quilici S (2002) Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bulletin of Entomological Research* **92**: 461-469.

Duyck PF, David P, Quilici S (2004) A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecological Entomology* **29**: 511-520.

Duyck PF, David P, Quilici S (2006) Climatic niche partitioning following successive invasions by fruit flies in La Réunion. *Journal of Animal Ecology* **75**: 518-526.

Earl DA, Von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**: 359-361.

Ekesi S, Maniania NK, Lux SA (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Science & Technology* **12**: 7-17.

Ekesi S, Billah MK (2007) A field guide to the management of economically important Tephtitid fruit flies in Africa. Icipe Science press, Kenya.

Ellers J, Boggs CL (2002) The evolution of wing color in *Colias* butterflies: heritability, sex linkage, and population divergence. *Evolution* **56**: 836-840.

Enkerlin D, Garcia LR, Fidel Lopez M (1989) Mexico, Central and South America. In: Fruit flies: their biology, natural enemies and control. Elsevier Science, Amsterdam.

Epsky ND, Heath RR, Guzman A, Meyer WL (1995) Visual cue and chemical cue interactions in a dry trap with food-based synthetic attractant for *Ceratitis capitata* and *Anastrepha ludens* (Diptera: Tephritidae). *Enviromental Entomology* **24**:1387-1395 (1995).

Epsky ND, Espinoza HR, Kendra PE, Abernathy R, Midgarden D, Heath RR (2010) Effective sampling of a synthetic protein-based attractant for *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology* **103**: 1886-1895.

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* **19**: 4113-4130.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611-2620.

Excoffier L, Lischer HEL (2010) Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564-567.

Fabre F, Ryckewaert P, Duyck PF, Chiroleu F, Quilici S (2003) Comparison of the efficacy of different food attractants and their concentration for melon fly (Diptera: Tephritidae). *Journal of Economic Entomology* **96**: 231-238.

Fairbairn DJ, Blanckenhorn W, Szekely T (2007) Sex, size and gender roles: Evolutionary studies of sexual size dimorphism**.** Oxford University Press, Oxford.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**: 1567-1587.

Fay HA, Drew RAI, Lloyd AC (1997) The eradication program for papaya fruit fly (*Bactrocera papayae* Drew and Hancock) in north Queensland. In: Allwood AJ, Drew RAI, eds. Management of fruit flies in the Pacific. ACIAR Proceedings, Canberra, Australia: ACIAR **76**: 259-261.

Fimiani P (1989) Mediterranean region. *Fruit flies: their biology, natural enemies and control.* Elsevier Science Publishers.

Fitzpatrick BM, Fordyce JA, Niemiller ML, Reynolds RG (2012) What can DNA tell us about biological invasions?. *Biological Invasions* **14**: 245-253.

Foucaud J, Orivel J, Loiseau A, Delabie JH, Jourdan H, Konghouleux D, Vonshal M, Tindo M, Mercier J-L, Fresneau D, Mikissa B, McGlynn T, Mikheyev AS, Oettler J, Estoup A (2010) Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evolutionary Applications* **3**: 363-374.

Francoy TM, Silva RAO, Nunes-Silva P, Menezes C, Imperatriz-Fonseca VL (2009) Gender identification of five genera of stingless bees (Apidae, Meliponini) based on wing morphology. *Genetics & Molecular Research* **8**: 207-214.

Frankham R (2005) Genetics and extinction. *Biological Conservation* **126**: 131-140.

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* **80**: 45-72

Garnier S, Alibert P, Audiot P, Prieur B, Rasplus JY (2004) Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. *Molecular Ecology* **13**: 1883-1897.

Gasperi G, Guglielmino CR, Malacrida AR, Milani R (1991) Genetic variability and gene flow in geographical populations of *Ceratitis capitata* (Wied.) (medfly). *Heredity* **67**: 347-356.

Gasperi G, Bonizzoni M, Gomulski LM, Murelli V, Torti C, Malacrida AR, Guglielmino CR (2002) Genetic differentiation, gene flow and the origin of infestations of the medfly, *Ceratitis capitata*. *Genetica* **116**: 125-135.

Gaston KJ, Jones AG, Hänel C, Chown SL (2003) Rates of species introduction to a remote oceanic island. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**: 1091-1098.

Gidaszewski NA, Baylac M, Klingenberg CP (2009) Evolution of sexual dimorphism of wing shape in the *Drosophila melanogaster* subgroup. *BMC Evolutionary Biology* **9**: 110-120.

Gilchrist AS (2004) Use of Microsatellite Tracking to Determine the Source of Queensland Fruit Fly Outbreaks in the Fruit Fly Exclusion Zone. Horticulture Australia Ltd, Sydney, Australia.

Gilchrist AS, Crisafulli DCA (2006) Using variation in wing shape to distinguish between wild and mass-reared individuals of Queensland fruit fly, *Bactrocera tryoni*. *Entomologia Experimentalis et Applicata* **119**: 175-178.

Gilchrist AS, Dominiak B, Gillespie PS, Sved JA (2006) Variation in population structure across the ecological range of the Queensland fruit fly, *Bactrocera tryoni*. *Australian Journal of Zoology* **54**: 87-95.

Gilchrist GW, Jeffers LM, West B, Folk DG, Suess J, Huey RB (2008) Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura*. *Evolutionary Applications* **1**: 513-523.

Gilchrist AS, Meats AW (2009) The genetic structure of populations of an invading pest fruit fly, *Bactrocera tryoni*, at the species climatic range limit. *Heredity* **105**: 165-172.

Goble TA, Dames JF, Hill MP, Moore SD (2011) Investigation of native isolates of entomopathogenic fungi for the biological control of three citrus pests. *Biocontrol Science & Technology* **21**: 1193-1211.

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. *Science* **327**: 812-818.

Gomulski LM, Bourtzis K, Brogna S, Morandi PA, Bonvicini C, Sebastiani F, Torti C, Guglielmino CR, Savakis C, Gasperi G, Malacrida AR (1998) Intron size polymorphism of the Adh(1) gene parallels the worldwide colonization history of the Mediterranean fruit fly, *Ceratitis capitata*. *Molecular Ecology* **7**: 1729-1741.

Grant WS, Little RM (1992) How sedentary are Greywing Francolins (*Francolinus africanus*)? *Evolution* **46**: 1477-1491.

Gregory PJ, Ingram JS, Brklacich M (2005) Climate change and food security. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**: 2139-2148.

Grout TG, Stoltz KC (2007) Developmental rates at constant temperatures of three economically important *Ceratitis* spp. (Diptera: Tephritidae) from Southern Africa. *Environmental Entomology* **36**: 1310-1317.

Grout TG, Daneel JH, Ware AB, Beck RR (2011) A comparison of monitoring systems used for *Ceratitis* species (Diptera: Tephritidae) in South Africa. *Crop Protection* **30**: 617-622.

Guillemaud T, Beaumont MA, Ciosi M, Cornuet JM, Estoup A (2010) Inferring introduction routes of invasive species using approximate Bayesian computation on microsatellite data. *Heredity* **104**: 88-99.

Gutierrez AP, Ponti L, Gilioli G (2014) Comments on the concept of ultra-low, cryptic tropical fruit fly populations. *Proceedings of the Royal Society B* **281**, available online at doi: 10.1098/rspb.2013.2825.

Handley L J.L, Estoup A, Evans DM, Thomas CE, Lombaert E, Facon B, Aebi A, Roy HE (2011) Ecological genetics of invasive alien species. *Biological Control* **56**: 409-428.

Hansen J, Sato M, Ruedy R (2012) Perception of climate change. *Proceedings of the National Academy of Sciences* **109,** available online at doi: 10.1073/pnas.1205276109.

Harris EJ (1989) Hawaiian islands and north America. *Fruit Flies: Their Biology, Natural Enemies and Control.* Elsevier Science Publishers.

Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumara) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science* **67**: 1352-1357.

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success?. *Biological Invasions* **10**: 483-506.

Higgins SI, Richardson DM (2014) Invasive plants have broader physiological niches. *Proceedings of the National Academy of Sciences* **111**: 10610-10614.

Hooper GHS, Drew RAI (1989) Australia and South Pacific islands. *Fruit flies: their biology, natural enemies and control.* Elsevier Science Publishers.

Hu J, Zhang J, Nardi F, Zhang R (2008) Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae), from China and Southeast Asia. *Genetica* **134**: 319324.

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* **46**: 10-18.

Irlich UM, Terblanche JS, Blackburn TM, Chown SL (2009) Insect rate-temperature relationships: environmental variation and the metabolic theory of ecology. *The American Naturalist* **174**: 819-835.

Jacquard C, Virgilio M, David P, Quilici S, De Meyer M, Delatte H (2013) Population structure of the melon fly, *Bactrocera cucurbitae*, in Reunion Island. *Biological invasions* **15**: 759-773.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.

Jessup AJ, Dominiak B, Woods B, De Lima CPF, Tomkins A, Smallridge CJ (2007) Area-wide management of fruit flies in Australia. In *Area-Wide Control of Insect Pests*. Springer, Netherlands.

Karsten M, Jansen van Vuuren B, Barnaud A, Terblanche JS (2013) Population genetics of *Ceratitis capitata* in South Africa: Implications for dispersal and pest management. *PLoS one* **8**, available online at doi: 10.1371/journal.pone.0054281.

Katsoyannos P (1992) Olive pests and their control in the Near East. *FAO plant production and protection paper*, (115).

Khamis FM, Karam N, Ekesi S, De Meyer M, Bonomi A, Gomulski LM, Scolari F, Gabrieli P, Siciliano P, Masiga D, Kenya EU, Gasperi G, Malacrida AR, Guglielmino CR (2009) Uncovering the tracks of a recent and rapid invasion: the case of the fruit fly pest *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Molecular Ecology* **18**: 4798-4810.

Kimberling DN (2004) Lessons from history: predicting successes and risks of intentional introductions for arthropod biological control. *Biological Invasions* **6**: 301-318.

Kirk H, Freeland JR (2011) Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences* **12**: 3966-3988.

Kirk H, Dorn S, Mazzi D (2013) Molecular genetics and genomics generate new insights into invertebrate pest invasions. *Evolutionary Applications* **6**: 842-856.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**: 353-357.

Koenig WD, Van Vuren D, Hooge PH (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution* **11**: 514-517.

Kokko H, López-Sepulcre A (2006) From individual dispersal to species ranges: perspectives for a changing world. *Science* **313**: 789-791.

Kourti A (2004) Estimates of gene flow from rare alleles in natural populations of medfly *Ceratitis capitata* (Diptera: Tephritidae). *Bulletin of Entomological Research* **94**: 449-456.

Koyama J, Kakinohana H, Miyatake T (2004) Eradication of the melon fly, *Bactrocera cucurbitae*, in Japan: importance of behaviour, ecology, genetics, and evolution. *Annual Review of Entomology* **49**: 331-349.

Krosch MN, Schutze MK, Armstrong KF, Boontop Y, Boykin LM, Chapman TA, Englezou A, Cameron SL, Clarke AR (2013) Piecing together an integrative taxonomic puzzle: microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis s.l.* (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. *Systematic Entomology* **38**: 2-13.

Lance DR, McInnis D (2005) Biological basis of the sterile insect technique. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique. Principles and Practice in Area Wide Integrated Pest Management, Springer.

Lande R (1995) Mutation and conservation. *Conservation Biology* **9**: 782-791.

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences* **104**: 3883-3888.

Le Roux J, Wieczorek AM (2009) Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Annals of Applied Biology* **154**: 1-17.

Leung B, Lodge DM, Finnoff D, Shogren JF, Lewis MA, Lamberti G (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**: 2407-2413.

Liebhold AM, Work TT, McCullough DG, Cavey JF (2006) Airline baggage as a pathway for alien insect species invading the United States. *American Entomologist* **52**: 48-54.

Lloret F, Médail F, Brundu G, Camarda I, Moragues E, Rita J, Lambdon P, Hulme PE (2005) Species attributes and invasion success by alien plants on Mediterranean islands. *Journal of Ecology* **93**: 512-520.

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* **20**: 223-228.

Lockwood JL, Cassey P, Blackburn TM (2009) The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity & Distributions* **15**: 904-910.

Lux SA, Copeland RS, White IM, Manrakhan A, Billah MK (2003) A new invasive fruit fly species from the *Bactrocera dorsalis* (Hendel) group detected in East Africa. *International Journal of Tropical Insect Science* **23**: 355-361.

Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. *American Naturalist* **146**: 489-518.

Maddison PA, Bartlett BJ (1989) A contribution towards the zoogeography of the Tephritidae. Fruit Flies: their biology, natural enemies and control. Elsevier Science Publishers.

Magniez-Jannin F, David B, Dommergues JL, Su ZH, Okada TS, Osawa S (2000) Analysing disparity by applying combined morphological and molecular approaches to French and Japanese carabid beetles. *Biological Journal of the Linnean Society* **7**: 343-358.

Malacrida AR, Guglielmino CR, Gasperi G, Baruffi L, Milani R (1992) Spatial and temporal differentiation in colonizing populations of *Ceratitis capitata*. *Heredity* **69**: 101-111.

Malacrida AR, Marinoni F, Torti C, Gomulski LM, Sebastiani F, Bonvicini C, Gasperi G, Guglielmino CR (1998) Genetic aspects of the worldwide colonization process of *Ceratitis capitata*. *Heredity* **89**: 501-507.

Malacrida AR, Gomulski LM, Bonizzoni M, Bertin S, Gasperi G, Guglielmino CR (2007) Globalization and fruitfly invasion and expansion: the medfly paradigm. *Genetica* **131**: 1-9.

Malan AP, Manrakhan A (2009) Susceptibility of the Mediterranean fruit fly (*Ceratitis capitata*) and the Natal fruit fly (*Ceratitis rosa*) to entomopathogenic nematodes. *Journal of Invertebrate Pathology* **100**: 47-49.

Mallet J, Joron M (1999) Evolution of diversity in warning coloration and mimicry: polymorphisms, shifting balance, and speciation. *Annual Review of Ecology & Systematics* **30**: 201-233.

Manrakhan A, Kotze C (2008) Attraction of *Ceratitis capitata*, *C. rosa* and *C. cosyra* (Diptera: Tephritidae) to proteinaceous baits. *Journal of Applied Entomology* **135**: 98-105.

Manrakhan A, Kotze C (2011) Attraction of *Ceratitis capitata*, *C. rosa* and *C. cosyra* (Diptera: Tephritidae) to proteinaceous baits. *Journal of Applied Entomolog y* **135**: 98-105.

Manrakhan A, Hattingh V, Venter JH, Holtzhausen M (2011) Eradication of *Bactrocera invadens* (Diptera: Tephritidae) in Limpopo Province, South Africa. *African Entomology* **19**: 650-659.

Manrakhan A, Addison P (2014) Assessment of fruit fly (Diptera: Tephritidae) management practices in deciduous fruit growing areas in South Africa. *Pest Management Science* **70**: 651-660.

Marsteller S, Adams DC, Collyer ML, Condon M (2009) Six cryptic species on a single species of host plant: morphometric evidence for possible reproductive character displacement. *Ecological Entomology* **34**: 66-73.

Mau RFL, Jang EB, Vargas RI (2007) The Hawaii area-wide fruit fly pest management programme: influence of partnerships and a good education programme. In *Area-Wide Control of Insect Pests*. Springer, Netherlands.

Mazzi D, Dorn S (2012) Movement of insect pests in agricultural landscapes. *Annals of Applied Biology* **160**: 97-113.

McPheron BA, Steck GJ (1996) Fruit Fly Pests: A world assessment of their biology and management. St. Lucie Press.

Meats A, Smallridge CJ (2007) Short- and long-range dispersal of medfly, *Ceratitis capitata* (Dipt., Tephritidae), and its invasive potential. *Journal of Applied Entomology* **131**: 518-523.

Meixner MD, McPheron BA, Silva JG, Gasparich GE, Sheppard WS (2002) The Mediterranean fruit fly in California: evidence for multiple introductions and persistent populations based on microsatellite and mitochondrial DNA variability. *Molecular Ecology* **11**: 891-899.

Meyerson LA, Mooney HA (2007) Invasive alien species in an era of globalization. *Frontiers in Ecology & the Environment* **5**: 199-208.

Mwatawala MW, White IM, Maerere AP, Senkondo FJ, De Meyer M (2004) A new invasive Bactrocera species (Diptera: Tephritidae) in Tanzania: short communication. *African Entomology* **12**: 154.

Nardi F, Carapelli A, Dallai R, Roderick GK, Frati F (2005) Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). *Molecular Ecology* **14**: 2729-2738.

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* **19**: 153-170.

Nice CC, Shapiro AM (1999) Molecular and morphological divergence in the butterfly genus *Lycaeides* (Lepidoptera: Lycaenidae) in North America: evidence of recent speciation. *Journal of Evolutionary Biology* **12**: 936-950.

Nieminen M, Singer MC, Fortelius W, Schöps K, Hanski I (2001) Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *The American Naturalist* **157**: 237-244.

Novak SJ (2007) The role of evolution in the invasion process. *Proceedings of the National Academy of Sciences* **104**: 3671-3672.

Nyamukondiwa C, Terblanche JS (2009) Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and feeding status. *Journal of Thermal Biology* **34**: 406-414.

Nyamukondiwa C, Kleynhans E, Terblanche JS (2010) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology* **35**: 565-575.

Nyamukondiwa C, Weldon CW, Chown SL, Le Roux PC, Terblanche JS (2013) Thermal biology, population fluctuations of temperature extremes for the management of two globally significant insect pests. *Journal of Insect Physiology* **59**: 1199-1211.

Nylin S, Wickman P-O, Wiklund C (1994) Genetics of development time in a butterfly: redictions from optimality and a test by subspecies crossings. *Proceedings of the Royal Society of London B* **257**: 215-19.

Oke OA (2008) Effectiveness of two insecticides to control melon fruit fly (*Bactrocera cucurbitae* Coq.) in cucumber (*Cucumis sativus* L). crop at Anse Boileau Seychelles. *European Journal of Scientific Research* **22:** 84-86.

Orian AJE, Moutia LA (1960). Fruit flies (Trypetidae) of economic importance in Mauritius. *Revue Agricole et Sucrière de l'Ile Maurice* **39**: 142-150.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* **13**: 55-65.

Papadopoulos NT, Plant RE, Carey JR (2013) From trickle to flood: the large-scale, cryptic invasion of California by tropical fruit flies. *Proceedings of the Royal Society B* **280**, available online at doi: 10.1098/rspb.2013.1466.

Pascual M, Chapuis MP, Mestres F, Balanya J, Huey RB, Gilchrist GW, Serra L, Estoup A (2007) Introduction history of *Drosophila subobscura* in the New World: a microsatellite-based survey using ABC methods. *Molecular Ecology* **16**: 3069-3083.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288-295.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* **28**: 2537-2539.

Pedigo LP (2002) Pest Management Theory in Entomology and Pest Management. Prentice Hall.

Peñarrubia-María IE, Quilici S, Schmitt C, Escudero-Colomar LA (2014) Evaluation of candidate systems for mass trapping against *Ceratitis* spp. on La Réunion island. *Pest Management Science* **70**: 448-453.

Perrings C, Dehnen-Schmutz K, Touza J, Williamson M (2005) How to manage biological invasions under globalization. *Trends in Ecology & Evolution* **20**: 212-215.

Peterson MA, Denno RF (1998) The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *The American Naturalist* **152**: 428-446.

Philibert A, Desprez-Loustau ML, Fabre B, Frey P, Halkett F, Husson C, Lung-Escarmant B, Marçais B, Robin C, Vacher C, Makowski D (2011). Predicting invasion success of forest pathogenic fungi from species traits. *Journal of Applied Ecology* **48**: 1381-1390.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS 2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536-539.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.

Pyšek P, Richardson DM, Pergl J, Jarošík V, Sixtová Z, Weber E (2008) Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution* **23**: 237-244.

Qvarnström A, Bailey RI (2008) Speciation through evolution of sex-linked genes. *Heredity* **102**: 4-15.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences* **94**: 9197-9201.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* **49**: 1280-1283.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, available online at http://www.R-project.org/.

Rey O, Estoup A, Vonshak M, Loiseau A, Blanchet S, Calcaterra L, Chifflet L, Rossi J-P, Kergoat GJ, Foucaud J, Orivel J, Maurice L, Schultz T, Facon B (2012) Where do adaptive shifts occur during invasion? A multidisciplinary approach to unravelling cold adaptation in a tropical ant species invading the Mediterranean area. *Ecology Letters* **15**: 1266-1275.

Reyes A, Ochando MD (2004) Mitochondrial DNA variation in Spanish populations of *Ceratitis capitata* (Wiedemann) (Tephritidae) and the colonization process. *Journal of Applied Entomology* **12**: 358-364.

Rice RE (2000) Bionomics of the olive fruit fly Bactrocera (Dacus) oleae. *UC Plant Protection Quarterly*, *10*(3), 1-5.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. *Trends in Ecology & Evolution* **8**: 129-132.

Rohlf FJ (1999) Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification* **16**: 197-223.

Rohlf FJ (2005) tpsDig, digitize landmarks and outlines, version 2.05. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**: 137-138.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**: 103-106.

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. *Annual Review of Ecology & Systematics* **32**: 305-332.

Santos M, Iriarte PF, Céspedes W, Balanyà J, Fontdevila A, Serra L (2004) Swift laboratory thermal evolution of wing shape (but not size) in *Drosophila subobscura* and its relationship with chromosomal inversion polymorphism. *Journal of Evolutionary Biology* **17**: 841-855.

Sar S, Balagawi S, Mararuai A, Putulan D (2001) Fruit Fly Research and Development in PNG. In Food security for Papua New Guinea. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference, PNG University of Technology, Lae, Papua New Guinea, 26-30 June 2000 (pp. 571-576).

Schierenbeck KA, Ellstrand NC (2009) Hybridization and the evolution of invasiveness in plants and other organisms. *Biological Invasions* **11**: 1093-1105.

Schutze MK, Krosch MN, Armstrong KF, Chapman TA, Englezou A, Chomič A, Cameron SL, Hailstones D, Clarke AR (2012a) Population structure of *Bactrocera dorsalis s.s.*, *B. papayae* and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. *BMC Evolutionary Biology* **12**: 130-143.

Schutze MK, Jessup A, Clarke AR (2012b) Wing shape as a potential discriminator of morphologically similar pest taxa within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae). *Bulletin of Entomological Research* **102**: 103-111.

Segura DF, Vera MT, Cagnotti CL, Vaccaro N, De Coll O, Ovruski SM, Cladera JL (2006) Relative abundance of *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) in diverse host species and localities of Argentina. *Annals of the Entomological Society of America* **99**: 70-83.

Shi W, Kerdelhue´ C, Ye H (2012) Genetic structure and inferences on potential source areas for *Bactrocera dorsalis* (Hendel) based on mitochondrial and microsatellite markers. *PLoS ONE* 7:e37083

Simberloff D (2009a) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics* **40**: 81-102.

Simberloff D (2009b) We can eliminate invasions or live with them. Successful management projects. *Biological Invasions* **11**: 149-157.

Skaife SH (1953) African Insect Life. Longmans, Green and co. Ltd., London.

Smith ESC, Price NS, Seewooruthun I (2000) Detection and eradication of the exotic fruit fly Bactrocera philippinensis from Australia. In *Proceedings of the Indian Ocean Commission, Regional Fruit Fly Symposium, Flic en Flac, Mauritius, 5th-9th June, 2000.* (pp. 201-205). Indian Ocean Commission.

Stephens AEA, Kriticos DJ, Leriche A (2007) The current and future potential geographical distribution of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *Bulletin of Entomological Research* **97**: 369-378.

Stohlgren TJ, Schnase JL (2006) Risk analysis for biological hazards: what we need to know about invasive species. *Risk Analysis* **26**: 163-173.

Storey JD (2002) A direct approach to false discovery rates. *Journal of the Royal Statistical Society, Series B* **64**: 479-498.

Strauss RE, Bookstein FL (1982) The truss: body form reconstructions in morphometrics. *Systematic Biology* **31**: 113-135.

Suckling DM, Jang EB, Holder P, Carvalho L, Stephens AEA (2008) Evaluation of lure dispensers for fruit fly surveillance in New Zealand. *Pest Management Science* **64**: 848-856.

Szűcs M, Melbourne BA, Tuff T, Hufbauer RA (2014) The roles of demography and genetics in the early stages of colonization. *Proceedings of the Royal Society B* **281**, available online at doi:10.1098/rspb.2014.1073.

Takezaki N, Nei M, Tamura K (2014) POPTREEW: Web version of POPTREE for constructing population trees from allele frequency data and computing some other quantities. *Molecular Biology & E*volution, available online at doi: 10.1093/molbev/msu093.

Toledo J, Liedo P, Flores S, Montoya P, Campos SE, Villasenor A (2006) Use of *Beauveria bassiana* and *Metarhizium anisopliae* for fruit fly control: a novel approach. In: Proceedings of the 7th International Symposium of Fruit flies of Economic Importance, pp. 127-132.

Vandewoestijne S, Baguette M (2004) Demographic versus genetic dispersal measures. *Population Ecology* **46**: 281-285.

Van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* **13**: 235-245.

Van Sauers-Muller A (1991) An overview of the carambola fruit fly *Bactrocera* species (Diptera: Tephritidae), found recently in Suriname. *Florida Entomologist* **74:** 432-440.

Vargas RI, Stark JD, Kido MH, Ketter HM, Whitehand LC (2000) Methyl Eugenol and Cue-lure traps for suppression of male Oriental fruit flies and Melon flies (Diptera: Tephritidae) in Hawaii: effects of lure mixtures and weathering. *Journal of Economic Entomology* **93**: 81-87.

Vargas RI, Miller NW, Prokopy RJ (2002) Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins, phloxine B and spinosada. *Entomologia Experimentalis et Applicata* **102**: 273-282.

Vayssières JF, Rey JY, Traore´ L (2007) Distribution and host plants of *Bactrocera cucurbitae* in West and Central Africa. *Fruits* **62:** 391–396.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Vera MT, Rodriguez R, Segura DF, Cladera JL, Sutherst RW (2002) Potential geographical distribution of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), with emphasis on Argentina and Australia. *Environmental Entomology* **31**: 1009-1022.

Villablanca FX, Roderick GK, Palumbi SR (1998) Invasion genetics of the Mediterranean fruit fly: variation in multiple nuclear introns. *Molecular Ecology* **7**: 547-560.

Virgilio M, Backeljau T, Barr N, De Meyer M (2008) Molecular evaluation of nominal species in the *Ceratitis fasciventris, C. anonae, C. rosa* complex (Diptera: Tephritidae). *Molecular Phylogenetics & Evolution* **48**: 270-280.

Virgilio M, Delatte H, Backeljau T, De Meyer M (2010) Macrogeographic population structuring in the cosmopolitan agricultural pest *Bactrocera cucurbitae* (Diptera: Tephritidae). *Molecular Ecology* **19**: 2713-2724.

Virgilio M, Delatte H, Quilici S, Backeljau T, De Meyer M (2013) Cryptic diversity and gene flow among three African agricultural pests : *Ceratitis rosa, Ceratitis fasciventris* and *Ceratitis anonae* (Diptera, Tephritidae). *Molecular Ecology* **22**: 2526-2539.

Vreysen MJB, Robinson AS, Hendrichs J (2007) Area-wide control of insect pests. From research to field implementation. Springer.

Wagner DL, Van Driesche RG (2010) Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology* **55**: 547-568.

Wan XW, Nardi F, Zhang B, Liu YH (2011) The oriental fruit fly, Bactrocera dorsalis, in China: origin and gradual inland range expansion associatedwith population growth. *PLoSONE* 6:e25238

Wan X, Liu Y, Zhang B (2012) Invasion history of the oriental fruit fly, Bactrocera dorsalis, in the Pacific-Asia region: two main invasion routes. *PLoS ONE* 7:e36176

Weber KE (1990) Selection on wing allometry in *Drosophila melanogaster*. *Genetics* **126**: 975-989.

Weber K, Eisman R, Morey L, Patty A, Sparks J, Tausek M, Zeng ZB (1999) An analysis of polygenes affecting wing shape on chromosome 3 in *Drosophila melanogaster*. *Genetics* **153**: 773-786.

Weems HV, Heppner JB, Fasulo TR Nation JL (2001) Caribbean fruit fly, Anastrepha suspensa (Loew) (Insecta: Diptera: Tephritidae). EENY-196, Featured Creatures from the Entomology and Nematology Department, Florida Cooperative Extension Service, Insitute of Food and Agricultural Sciences. University of

White IM, Elson-Harris MM (1994) Fruit flies of economics significance: Their identification and bionomics. ACIAR, CAB International, Wallingford Oxon.

White IM, De Meyer M, Stonehouse J (2001) A review of the native and introduced fruit flies (Diptera: Tephritidae) in the Indian Ocean Islands of Mauritius, Reunion, Rodrigues and Seychelles. In: Proceedings of the Indian Ocean Commission Regional Fruit Fly Symposium, pp.15-21.

Wicker-Thomas C (2007) Pheromonal communication involved in courtship behavior in Diptera. *Journal of Insect Physiology* **53**: 1089-1100.

Wilson JR, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution* **24**: 136-144.

Yu H, Frommer M, Robson MK, Meats AW, Shearman DCA, Sved JA (2001) Microsatellite analysis of the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae) indicates spatial structuring: implications for population control. *Bulletin of Entomological Research* **91**: 139-147.

Zelditch ML, Lundrigan BL, Garland T (2004) Developmental regulation of skull morphology. I. Ontogenetic dynamics of variance. *Evolution & Development* **6**: 194-206.

Zimmerman E, Palsson A, Gibson G (2000) Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics* **155**: 671-683.

Zygouridis NE, Augustinos AA, Zalom FG, Mathiopoulos KD (2008) Analysis of olive fly invasion in California based on microsatellite markers. *Heredity* **102**: 402-412.

APPENDICES

APPENDIX I: List of hosts (including those that are heavily infested, occasionally infested, rarely infested and where infestation needs to be confirmed) used by *Ceratitis capitata*. The list is adapted from White & Elson-Harris 1994 and CABI Invasive species compendium see www.cabi.org (7 August 2014).

Acca sellowiana

Acokanthera oppositifolia

Acokanthera ouabaio

Actinidia deliciosa (kiwifruit)

Anacardium occidentale (cashew nut)

Ananas comosus (pineapple)

Annona cherimola (cherimoya)

Annona glabra (pondapple)

Annona muricata (soursop)

Annona reticulate (bullock's heart)

Annona squamosa (sugar apple)

Antidesma dallachiana

Antidesma venosum

Arbutus unedo (arbutus)

Arenga pinnata (sugar palm)

Argania spinosa (argan tree)

Argemone mexicana (Mexican prickle poppy)

Artabotrys hexapetalus (fragrant tailgrape)

Artocarpus altilis (breadfruit)

Asimina obovata (bigflower pawpaw)

Asimina parviflora (smallflower pawpaw)

Asimina pygmaea (sprawling pawpaw)

Asimina reticulata (seminoletea pawpaw)

Asimina triloba (pawpaw)

Asparagus densiflorus (Sprenger asparagus)

Atropa belladonna (belladonna)

Averrhoa bilimbi (blimbe)

Averrhoa carambola (carambola)

Azima tetracantha (beehanger)

Banksia prionotes

Berberis holstii (barberry)

Blighia sapida (akee)

Brucea antidysenterica

Brucea ferruginea

Bumelia lycioides (buckthorn bumelia)

Bumelia tenax (buckthorn)

Butia eriospatha

Calophyllum inophyllum (beauty-leaf)

Calophyllum tacamahaca

Cananga odorata (perfume tree)

Capparis citrifolia (caper)

Capparis sepiaria (indian caper)

Capsicum annuum (bell pepper)

Capsicum frutescens (chilli)

Carica papaya (papaw)

Carissa bispinosa (hedge thorn)

Carissa carandas (caranda (plum))

Carissa edulis (egyptian carissa)

Carissa grandiflora (natal plum)

Carissa macrocarpa (natal plum)

Carica quercifolia (dwarf papaya)

Carya illinoinensis (pecan)

Casimiroa edulis (white sapote)

Cestrum (night-blooming jasmine)

Chrysophyllum africanum (African star apple)

Chrysophyllum cainito (star apple)

Chrysobalanus ellipticus

Chrysobalanus icaco (icaco plum)

Chrysophyllum cainito (caimito)

Chrysophyllum oliviforme

Chrysophyllum polynesicum

Chrysophyllum viridifolium

Cinnamomum verum (cinnamon)

Citharexylum fruticosum (Florida fiddlewood)

Citrus aurantiifolia (lime)

Citrus aurantium (sour orange)

Citrus limetta (sweet lemon tree)

Citrus limon (lemon)

Citrus limonia (mandarin lime)

Citrus maxima (pummelo)

Citrus medica (citron)

Citrus nobilis (tangor)

Citrus paradisi (pomelo)

Citrus reticulata (mandarin)

Citrus reticulata x paradisi (tangelo)

Citrus sinensis (navel orange)

Citrus x paradisi (grapefruit)

Clausena anisata (horsewood)

Clausena lansium (Chinese wampee)

Clintonia umbellulata (spreckled beadlily)

Coccoloba uvifera (seaside grape)

Coffea (coffee)

Coffea arabica (arabica coffee)

Coffea canephora (robusta coffee)

Coffea liberica (Liberian coffee tree)

Cola natalensis

Cotoneaster adpressus (early creeping cotoneaster)

Crataegus azarolus (hawthorn)

Crateva tapia

Crinum asiaticum (St. John's lily)

Cucumis dipsaceus (hedgehog gourd)

Cucumis sativus (cucumber)

Cucurbita maxima (hubbard squash)

Cucurbita moschata (winter crookneck pumpkin)

Cucurbita pepo (vegetable marrow)

Cydonia oblonga (quince)

Cyphomandra betacea (tree tomato)

Cyphomandra crassicaulis (tree tomato)

Dimocarpus longan (longan tree)

Diospyros (malabar ebony)

Diospyros abyssinica

Diospyros kaki (persimmon)

Diospyros mespiliformis (ebony diospiros)

Diospyros pallens

Diospyros virginiana (persimmon (common))

Dovyalis caffra (kei apple)

Dovyalis hebecarpa (ketembilla)

Drypetes natalensis

Durio zibethinus (durian)

Ehretia cymosa

Ekebergia capensis (dog plum)

Englerophytum magalismontanum

Eriobotrya japonica (loquat)

Euclea divinorum

Eugenia brasiliensis (Spanish cherry)

Eugenia dombeyi (brazil cherry)

Eugenia paniculata

Eugenia uniflora (surinam cherry)

Euphorbia lathyris (gopher apple)

Euphoria longan (longan)

Feijoa sellowiana (Horn of plenty)

Ficus carica (fig)

Filicium decipiens

Flacourtia indica (governor's plum)

Flagellaria guineensis

Flueggea virosa

Fortunella (kumquats)

Fortunella japonica (round kumquat)

Fortunella margarita (megami kumquat)

Garcinia livingstonei (african mangosteen)

Garcinia mangostana (mangosteen)

Garcinia xanthochymus (gourka)

Gardenia sp.

Glycosmis pentaphylla (Malay glycosmis)

Gossypium sp.

Guettarda speciosa

Harpephyllum caffrum

Hevea brasiliensis (Brazil rubber)

Homalocladium platycladum (ribbon bush)

Hylocereus undatus (dragon fruit)

Ilex vomitoria (yaupon)

Juglans hindsii

Juglans regia (walnut)

Landolphia sp

Latania loddigesii (blue palm)

Litchi chinensis (litchi)

Lycium (boxthorn)

Lycium barbarum (Matrimonyvine)

Lycium carolinanum (boxthorn)

Lycium chinense (boxthorn)

Lycium europaeum (european boxthorn)

Lycium horridum (African buckthorn)

Lycopersicon esculentum (tomato)

Maclura pomifera (osage orange)

Malpighia glabra (acerola)

Malpighia punicifolia (acerola)

Malus domestica (apple)

Malus floribunda

Malus pumila (common apple)

Mammea americana (mammee apple)

Mangifera indica (mango)

Manilkara butugi

Manilkara sansibarensis

Manilkara zapota (sapodilla)

Marrubium vulgare (common hoarhound)

Mastichodendron foetidissimum (ironwood)

Melicoccus bijugatus (Spanish lime)

Melothria pendula (creeping cucumber)

Mespilus germanica (medlar)

Mimusops bagshawei

Mimusops caffra

Mimusops elengi (spanish cherry)

Mimusops fruticosa

Mimusops kirkii

Mimusops obtusifolia

Momordica balsamina (balsam apple)

Monstera deliciosa (ceriman)

Morus nigra (black mulberry)

Muntingia calabura (Jamaica cherry)

Murraya paniculata (orange jessamine)

Musa acuminata (Chinese banana)

Musa x paradisiaca (plantain)

Myrciaria cauliflora

Myrciaria edulis (willow-leaved eugenia)

Myrianthus arboreus

Nephelium lappaceum (rambutan)

Noronhia emarginata (Chinese plum)

Nyssa ogeche (ogeechee tupelo)

Nyssa sylvatica (sour gum)

Nyssa sylvatica (swamp black tupelo)

Ochrosia elliptica (ochrosia)

Olea europaea (olive)

Olea woodiana

Opilia amentacea

Opuntia ficus-indica (prickly pear)

Parmentiera aceleatan (cuachilote)

Passiflora coerulea (blue-crown passionflower)

Passiflora edulis (passionfruit)

Passiflora incarnata (maypop)

Passiflora laurifolia (Jamaica honeysuckle)

Passiflora lingularis (sweet granadilla)

Passiflora mollissima (soft-leaf passion flower)

Passiflora quadrangularis (giant granadilla)

Passiflora suberosa (corkystem passionflower)

Peponia mackennii (wild cucurbit)

Pereskia aculeata (Lemon-vine)

Persea americana (avocado)

Phaseolus limensis (lima bean)

Phaseolus lunatus (cibet bean)

Phaseolus vulgaris (string bean)

Phoenix dactylifera (date-palm)

Phyllantus acidus (Ceylon gooseberry)

Physalis peruviana (Cape gooseberry)

Pimenta dioica (pimenta)

Pithecollobium dulce

Pleiogynium cerasiferum (burdekin plum)

Podocarpus elongatus (african yellow wood)

Poncirus trifoliata (trifoliate orange)

Pouteria caimito

Pouteria campechiana (egg fruit)

Pouteria sapota (mammey sapote)

Pouteria viridis (green sapote)

Prunus (stone fruit)

Prunus armeniaca (apricot)

Prunus avium (sweet cherry)

Prunus cerasus (sour cherry)

Prunus domestica (plum)

Prunus dulcis (almond)

Prunus japonica (plum)

Prunus persica (peach)

Prunus salicina (Japanese plum)

Prunus umbellata (flatwoods plum)

Psidium cattleianum (strawberry guava)

Psidium friedrichsthalianum (wild guava)

Psidium guajava (guava)

Psidium guineense (Brazilian guava)

Psidium littorale (yellow cattley guava)

Psidium longipes (strawberry guava)

Punica granatum (pomegranate)

Putranjiva roxburghii (India amulet plant)

Pyracantha coccinea (laland firethorn)

Pyrus communis (European pear)

Pyrus pyrifolia (Oriental pear tree)

Pyrus syriaca

Ribes sp. (currant, gooseberry)

Robinia sp.

Rosa sp. (rose)

Royena pallens (pale-branched royena)

Rubus idaeus (raspberry)

Rubus loganobaccus (loganberry)

Salix sp. (willow leaf)

Sandoricum koetjape (santol)

Santalum album (Indian sandalwood)

Santalum freycinetianum (beach sandalwood)

Scaevola plumieri

Scaevola sericea

Scaevola taccada (beach naupaka)

Schinus molle (California pepper tree)

Sechium edule (christophine)

Selenicereus pteranthus (cactus)

Serenoa repens (saw palmetto)

Severinia buxifolia (Chinese box orange)

Sideroxylon inerme

Smilax beyrichii

Solanum aculeatissimum (nightshade)

Solanum capsicastrum (false Jerusalem cherry)

Solanum carolinense (Caroline horse nettle)

Solanum incanum (grey bitter-apple)

Solanum lycopersicum (tomato)

Solanum macrocarpon (local garden egg)

Solanum mauritianum (tree tobacco)

Solanum melanocerasum (garden huckleberry)

Solanum melongena (aubergine)

Solanum muricatum (melon pear)

Solanum nigrum (black nightshade)

Solanum pseudocapsicum (Jerusalem-cherry)

Solanum seaforthianum (Brazilian nightshade)

Solanum sodomeum (apple of Sodom)

Sorbus sp.

Spondias cytherea

Spondias dulcis (otaheite apple)

Spondias mombin (cajamerin)

Spondias purpurea (red mombin)

Spondias tuberosa

Strychnos atherstonei

Strychnos decussata

Strychnos henningsii

Strychnos potatorum

Strychnos pungens

Syagrus campestris (field syagrus palm)

Synsepalum dulcificum

Syzygium cumini (black plum)

Syzygium jambos (rose apple)

Syzygium malaccense (malay-apple)

Syzygium samarangense (water apple)

Teclea trichocarpa

Terminalia catappa (Singapore almond)

Terminalia chebula (chebula terminalia)

Terminalia pallida

Theobroma cacao (cocoa)

Thevetia peruviana (exile tree)

Vaccinium cereum

Vaccinium corymbosum (blueberry)

Vangueria edulis (vangueria)

Vangueria infausta

Vepris lanceolata

Vicia faba (broad bean)

Vitis lambrusca (Isabella grape)

Vitis vinifera (grapevine)

Wikstroemia phillyreifolia (wikstroemia)

Ximenia americana (Hog plum)

Ziziphus joazeiro

Ziziphus jujuba (chinese date)

Ziziphus mauritiana (jujube)

APPENDIX II: List of hosts (including those that are heavily infested, occasionally infested, rarely infested and where infestation needs to be confirmed) used by *Ceratitis rosa*. The list is adapted from White & Elson-Harris 1994 and CABI Invasive species compendium see www.cabi.org (7 August 2014).

Acca sellowiana

Allophylus pervillei

Anacardium occidentale (cashew nut)

Angylocalyx braunii

Annona cherimola (cherimoya)

Annona muricata (soursop)

Annona reticulata (custard apple)

Annona senegalensis (wild custard apple)

Annona squamosa (sugar apple)

Averrhoa bilimbi (blimbe)

Averrhoa carambola (carambola)

Calophyllum tacamahaca

Calycosiphonia spathicalyx

Cananga odorata (perfume tree)

Capsicum frutescens (tobasco pepper)

Carica cauliflora

Carica papaya (papaya)

Carissa carandas (caranda (plum))

Carissa macrocarpa (natal plum)

Cereus peruvianus

Chrysophyllum cainito (caimito)

Chrysophyllum carpussum

Chrysophyllum natalense

Citrus aurantium (sour orange)

Citrus nobilis (tangor)

Citrus reticulata (mandarin)

Citrus sinensis (navel orange)

Citrus x paradisi (grapefruit)

Coccoloba uvifera (seaside grape)

Coffea arabica (arabica coffee)

Cola natalensis

Cucurbita (pumpkin)

Cydonia oblonga (quince)

Dictyophleba lucida

Dimocarpus longan (longan tree)

Diospyros kabuyeana

Diospyros kaki (persimmon)

Dovyalis caffra (kei apple)

Dovyalis hebecarpa (ketembilla)

Drypetes battiscombei

Drypetes natalensis

Drypetes natalensis var. leiogyna

Ehretia cymosa

Ekebergia capensis

Englerophytum magalismontanum

Englerophytum natalense

Eriobotrya japonica (loquat)

Eugenia uniflora (surinam cherry)

Feijoa sellowiana (Horn of plenty)

Ficus carica (fig)

Flacourtia indica (governor's plum)

Garcinia mangostana (mangosteen)

Harpephyllum caffrum

Hylocereus undatus (dragon fruit)

Inga laurina (Spanish oak)

Lettowianthus stellatus

Litchi chinensis (litchi)

Ludia mauritiana

Lycopersicon esculentum (tomatoe)

Malus domestica (apple)

Mangifera indica (mango)

Manilkara zapota (sapodilla)

Mimusops elengi (spanish cherry)

Monanthotaxis fornicata

Monodora grandidieri

Murraya paniculata (orange jessamine)

Musa acuminata (wild banana)

Myrianthus arboreus

Opilia amentacea

Opuntia ficus-indica (prickly pear)

Pachystela excelsa

Persea americana (avocado)

Phyllanthus acidus (star gooseberry)

Pithecellobium dulce (Manila tamarind)

Pouteria campechiana (canistel)

Pouteria usambarensis

Prunus armeniaca (apricot)

Prunus domestica (plum)

Prunus persica (peach)

Prunus salicina (Japanese plum)

Psidium cattleianum (strawberry guava)

Psidium friedrichsthalianum (wild guava)

Psidium guajava (guava)

Psidium guineense (Guinea guava)

Psidium japonicum

Psidium longipes (strawberry guava)

Pyrus communis (European pear)

Rawsonia lucida

Salacia elegans

Solanum giganteum

Solanum lycopersicum (tomato)

Solanum mauritianum (tree tobacco)

Sphaerocoryne gracilis

Strychnos henningsii

Strychnos spinosa

Synsepalum brevipes

Synsepalum dulcificum

Synsepalum subvertillatum

Syzygium aqueum (watery rose-apple)

Syzygium cumini (black plum)

Syzygium jambos (rose apple)

Syzygium malaccense (malay-apple)

Syzygium samarangense (water apple)

Terminalia catappa (Singapore almond)

Theobroma cacao (cocoa)

Toddalia asiatica

Tricalysia pallens

Uvaria acuminata

Uvaria lucida

Vitis vinifera (grapevine)

Ziziphus jujuba (common jujube)

Ziziphus mauritiana (jujube)

APPENDIX III: Frequency of null alleles calculated according to Dempster *et al*. (1977) in FREENA 1.0 (Chapuis & Estoup 2007) for 323 *Ceratitis capitata* individuals.

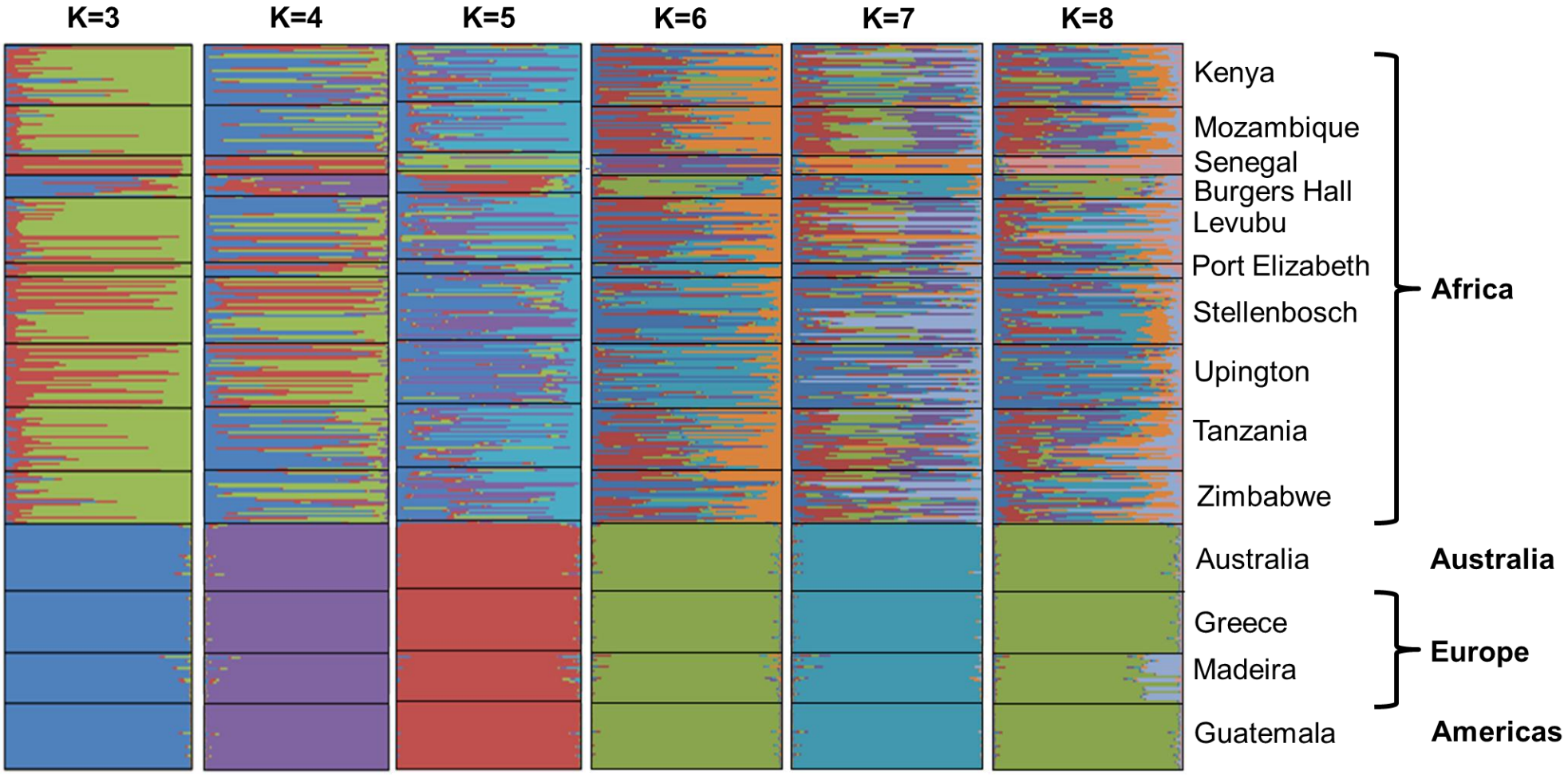
Locus	Population	Estimate of null allele frequency	Population	Average	SD	Locus	Average	SD
C1	Australia	0.060	Australia	0.064	0.095	C1	0.138	0.122
C1	Burgers Hall	0.000	Burgers Hall	0.023	0.053	C2	0.148	0.132
C1	Greece	0.000	Greece	0.070	0.112	C3	0.058	0.059
C1	Guatamala	0.000	Guatamala	0.047	0.070	C4	0.196	0.095
C1	Kenya	0.240	Kenya	0.139	0.087	C5	0.039	0.057
C1	Levubu	0.245	Levubu	0.115	0.103	C6	0.117	0.092
C1	Madeira	0.000	Madeira	0.125	0.101	C9	0.049	0.057
C1	Mozambique	0.256	Mozambique	0.115	0.110	C12	0.038	0.040
C1	Port Elizabeth	0.158	Port Elizabeth	0.068	0.090	C13	0.042	0.063
C1	Senegal	0.018	Senegal	0.061	0.107	C15	0.036	0.043
C1	Stellenbosch	0.180	Stellenbosch	0.099	0.100	C16	0.110	0.118
C1	Tanzania	0.264	Tanzania	0.095	0.106			
C1	Upington	0.164	Upington	0.098	0.083			
C1	Zimbabwe	0.342	Zimbabwe	0.116	0.117			
C2	Australia	0.043						
C2	Burgers Hall	0.000						
C2	Greece	0.000						
C2	Guatamala	0.000						
C2	Kenya	0.291						
C2	Levubu	0.209						
C2	Madeira	0.043						

C2	Mozambique	0.231
C2	Port Elizabeth	0.290
C2	Senegal	0.000
C2	Stellenbosch	0.288
C2	Tanzania	0.277
C2	Upington	0.261
C2	Zimbabwe	0.138
C3	Australia	0.005
C3	Burgers Hall	0.061
C3	Greece	0.034
C3	Guatamala	0.001
C3	Kenya	0.143
C3	Levubu	0.067
C3	Madeira	0.178
C3	Mozambique	0.104
C3	Port Elizabeth	0.000
C3	Senegal	0.119
C3	Stellenbosch	0.036
C3	Tanzania	0.000
C3	Upington	0.070
C3	Zimbabwe	0.000
C4	Australia	0.000
C4	Burgers Hall	0.174
C4	Greece	0.283
C4	Guatamala	0.204
C4	Kenya	0.248
C4	Levubu	0.255
C4	Madeira	0.145
C4	Mozambique	0.243
C4	Port Elizabeth	0.088
C4	Senegal	0.355
C4	Stellenbosch	0.109
C4	Tanzania	0.193
C4	Upington	0.139
C4	Zimbabwe	0.311
C5	Australia	0.165
C5	Burgers Hall	0.000
C5	Greece	0.000
C5	Guatamala	0.000
C5	Kenya	0.093
C5	Levubu	0.026
C5	Madeira	0.106
C5	Mozambique	0.117
C5	Port Elizabeth	0.000

C5	Senegal	0.001
C5	Stellenbosch	0.000
C5	Tanzania	0.000
C5	Upington	0.000
C5	Zimbabwe	0.044
C6	Australia	0.000
C6	Burgers Hall	0.000
C6	Greece	0.117
C6	Guatamala	0.000
C6	Kenya	0.169
C6	Levubu	0.238
C6	Madeira	0.175
C6	Mozambique	0.245
C6	Port Elizabeth	0.056
C6	Senegal	0.049
C6	Stellenbosch	0.248
C6	Tanzania	0.078
C6	Upington	0.098
C6	Zimbabwe	0.173
C9	Australia	0.000
C9	Burgers Hall	0.000
C9	Greece	0.001
C9	Guatamala	0.001
C9	Kenya	0.053
C9	Levubu	0.079
C9	Madeira	0.000
C9	Mozambique	0.000
C9	Port Elizabeth	0.082
C9	Senegal	0.000
C9	Stellenbosch	0.104
C9	Tanzania	0.074
C9	Upington	0.174
C9	Zimbabwe	0.113
C12	Australia	0.015
C12	Burgers Hall	0.000
C12	Greece	0.000
C12	Guatamala	0.101
C12	Kenya	0.072
C12	Levubu	0.023
C12	Madeira	0.064
C12	Mozambique	0.000
C12	Port Elizabeth	0.072
C12	Senegal	0.026
C12	Stellenbosch	0.000

C12	Tanzania	0.000
C12	Upington	0.115
C12	Zimbabwe	0.045
C13	Australia	0.000
C13	Burgers Hall	0.017
C13	Greece	0.000
C13	Guatamala	0.033
C13	Kenya	0.068
C13	Levubu	0.000
C13	Madeira	0.230
C13	Mozambique	0.000
C13	Port Elizabeth	0.000
C13	Senegal	0.108
C13	Stellenbosch	0.033
C13	Tanzania	0.000
C13	Upington	0.034
C13	Zimbabwe	0.059
C15	Australia	0.117
C15	Burgers Hall	0.000
C15	Greece	0.044
C15	Guatamala	0.032
C15	Kenya	0.111
C15	Levubu	0.002
C15	Madeira	0.105
C15	Mozambique	0.015
C15	Port Elizabeth	0.000
C15	Senegal	0.000
C15	Stellenbosch	0.010
C15	Tanzania	0.031
C15	Upington	0.005
C15	Zimbabwe	0.034
C16	Australia	0.299
C16	Burgers Hall	0.001
C16	Greece	0.289
C16	Guatamala	0.140
C16	Kenya	0.044
C16	Levubu	0.128
C16	Madeira	0.333
C16	Mozambique	0.054
C16	Port Elizabeth	0.001
C16	Senegal	0.000
C16	Stellenbosch	0.085
C16	Tanzania	0.128
C16	Upington	0.017
C16	Zimbabwe	0.017

APPENDIX IV Assignment results from STRUCTURE for K=3-8 for 14 *Ceratitis capitata* populations.



APPENDIX V: Estimates of null allele frequencies (for each locus in each population, averaged over populations and averaged over loci) of *Ceratitis rosa* calculated based on the EM algorithm implemented in the program FREENA (Chapuis & Estoup 2007).

Locus	Pop	Estimate of null allele frequency	Pop	Average	SD	Locus	Average	SD
FAR1	Bloemfontein	0.253	Bloemfontein	0.064	0.088	FAR1	0.272	0.073
FAR1	King Williams Town	0.225	King Williams Town	0.080	0.098	FAR2	0.025	0.033
FAR1	Kirkwood	0.396	Kirkwood	0.071	0.116	FAR3	0.106	0.073
FAR1	Komatiepoort	0.240	Komatiepoort	0.070	0.082	FAR4	0.057	0.063
FAR1	Louis Trichardt	0.341	Louis Trichardt	0.061	0.111	FAR6	0.038	0.057
FAR1	Lutzville	0.270	Lutzville	0.080	0.127	FAR8	0.202	0.098
FAR1	Mt. Edgecombe	0.157	Mt. Edgecombe	0.066	0.085	FAR11	0.020	0.041
FAR1	Nelspruit	0.211	Nelspruit	0.062	0.075	FAR12	0.037	0.049
FAR1	Nkwalini	0.124	Nkwalini	0.071	0.121	FAR14	0.010	0.017
FAR1	Onrus River	0.214	Onrus River	0.061	0.082	FAR15	0.036	0.057
FAR1	Paarl	0.241	Paarl	0.081	0.094	FAR16	0.046	0.055
FAR1	Pietermaritzburg	0.268	Pietermaritzburg	0.068	0.088			
FAR1	Piketberg	0.311	Piketberg	0.084	0.094			
FAR1	Pinetown	0.254	Pinetown	0.101	0.098			
FAR1	Port Elizabeth	0.322	Port Elizabeth	0.057	0.098			
FAR1	Potchefstroom	0.353	Potchefstroom	0.065	0.108			
FAR1	Pretoria	0.345	Pretoria	0.065	0.105			
FAR1	Riebeeck Kasteel	0.245	Riebeeck Kasteel	0.111	0.102			
FAR1	Rustenburg	0.251	Rustenburg	0.077	0.087			
FAR1	Somerset West	0.344	Somerset West	0.083	0.120			
FAR1	Stellenbosch	0.403	Stellenbosch	0.118	0.116			
FAR1	Tzaneen	0.207	Tzaneen	0.072	0.070			

FAR2	Bloemfontein	0.012
FAR2	King Williams Town	0.000
FAR2	Kirkwood	0.037
FAR2	Komatiepoort	0.000
FAR2	Louis Trichardt	0.000
FAR2	Lutzville	0.000
FAR2	Mt. Edgecombe	0.000
FAR2	Nelspruit	0.029
FAR2	Nkwalini	0.000
FAR2	Onrus River	0.028
FAR2	Paarl	0.000
FAR2	Pietermaritzburg	0.002
FAR2	Piketberg	0.092
FAR2	Pinetown	0.086
FAR2	Port Elizabeth	0.042
FAR2	Potchefstroom	0.097
FAR2	Pretoria	0.024
FAR2	Riebeeck Kasteel	0.000
FAR2	Rustenburg	0.000
FAR2	Somerset West	0.002
FAR2	Stellenbosch	0.068
FAR2	Tzaneen	0.039
FAR3	Bloemfontein	0.106
FAR3	King Williams Town	0.034
FAR3	Kirkwood	0.065
FAR3	Komatiepoort	0.172
FAR3	Louis Trichardt	0.056
FAR3	Lutzville	0.000
FAR3	Mt. Edgecombe	0.072
FAR3	Nelspruit	0.143
FAR3	Nkwalini	0.126
FAR3	Onrus River	0.126
FAR3	Paarl	0.092
FAR3	Pietermaritzburg	0.138
FAR3	Piketberg	0.081
FAR3	Pinetown	0.015
FAR3	Port Elizabeth	0.000
FAR3	Potchefstroom	0.000
FAR3	Pretoria	0.169
FAR3	Riebeeck Kasteel	0.168
FAR3	Rustenburg	0.157
FAR3	Somerset West	0.242
FAR3	Stellenbosch	0.248
FAR3	Tzaneen	0.131

FAR4	Bloemfontein	0.000
FAR4	King Williams Town	0.201
FAR4	Kirkwood	0.039
FAR4	Komatiepoort	0.000
FAR4	Louis Trichardt	0.204
FAR4	Lutzville	0.016
FAR4	Mt. Edgecombe	0.000
FAR4	Nelspruit	0.000
FAR4	Nkwalini	0.083
FAR4	Onrus River	0.159
FAR4	Paarl	0.056
FAR4	Pietermaritzburg	0.060
FAR4	Piketberg	0.000
FAR4	Pinetown	0.063
FAR4	Port Elizabeth	0.035
FAR4	Potchefstroom	0.006
FAR4	Pretoria	0.040
FAR4	Riebeeck Kasteel	0.093
FAR4	Rustenburg	0.103
FAR4	Somerset West	0.029
FAR4	Stellenbosch	0.070
FAR4	Tzaneen	0.000
FAR6	Bloemfontein	0.000
FAR6	King Williams Town	0.001
FAR6	Kirkwood	0.000
FAR6	Komatiepoort	0.000
FAR6	Louis Trichardt	0.000
FAR6	Lutzville	0.000
FAR6	Mt. Edgecombe	0.091
FAR6	Nelspruit	0.104
FAR6	Nkwalini	0.000
FAR6	Onrus River	0.000
FAR6	Paarl	0.048
FAR6	Pietermaritzburg	0.117
FAR6	Piketberg	0.019
FAR6	Pinetown	0.000
FAR6	Port Elizabeth	0.072
FAR6	Potchefstroom	0.021
FAR6	Pretoria	0.049
FAR6	Riebeeck Kasteel	0.222
FAR6	Rustenburg	0.000
FAR6	Somerset West	0.000
FAR6	Stellenbosch	0.000
FAR6	Tzaneen	0.082

FAR8	Bloemfontein	0.196
FAR8	King Williams Town	0.236
FAR8	Kirkwood	0.145
FAR8	Komatiepoort	0.100
FAR8	Louis Trichardt	0.000
FAR8	Lutzville	0.374
FAR8	Mt. Edgecombe	0.203
FAR8	Nelspruit	0.138
FAR8	Nkwalini	0.400
FAR8	Onrus River	0.197
FAR8	Paarl	0.245
FAR8	Pietermaritzburg	0.117
FAR8	Piketberg	0.302
FAR8	Pinetown	0.269
FAR8	Port Elizabeth	0.143
FAR8	Potchefstroom	0.145
FAR8	Pretoria	0.048
FAR8	Riebeeck Kasteel	0.232
FAR8	Rustenburg	0.185
FAR8	Somerset West	0.280
FAR8	Stellenbosch	0.316
FAR8	Tzaneen	0.179
FAR11	Bloemfontein	0.000
FAR11	King Williams Town	0.000
FAR11	Kirkwood	0.000
FAR11	Komatiepoort	0.126
FAR11	Louis Trichardt	0.000
FAR11	Lutzville	0.093
FAR11	Mt. Edgecombe	0.000
FAR11	Nelspruit	0.000
FAR11	Nkwalini	0.000
FAR11	Onrus River	0.000
FAR11	Paarl	0.000
FAR11	Pietermaritzburg	0.000
FAR11	Piketberg	0.000
FAR11	Pinetown	0.000
FAR11	Port Elizabeth	0.000
FAR11	Potchefstroom	0.000
FAR11	Pretoria	0.000
FAR11	Riebeeck Kasteel	0.050
FAR11	Rustenburg	0.000
FAR11	Somerset West	0.000
FAR11	Stellenbosch	0.116
FAR11	Tzaneen	0.053

FAR12	Bloemfontein	0.011
FAR12	King Williams Town	0.107
FAR12	Kirkwood	0.077
FAR12	Komatiepoort	0.065
FAR12	Louis Trichardt	0.000
FAR12	Lutzville	0.098
FAR12	Mt. Edgecombe	0.000
FAR12	Nelspruit	0.000
FAR12	Nkwalini	0.042
FAR12	Onrus River	0.057
FAR12	Paarl	0.159
FAR12	Pietermaritzburg	0.000
FAR12	Piketberg	0.049
FAR12	Pinetown	0.125
FAR12	Port Elizabeth	0.007
FAR12	Potchefstroom	0.001
FAR12	Pretoria	0.003
FAR12	Riebeeck Kasteel	0.021
FAR12	Rustenburg	0.000
FAR12	Somerset West	0.000
FAR12	Stellenbosch	0.000
FAR12	Tzaneen	0.000
FAR14	Bloemfontein	0.046
FAR14	King Williams Town	0.000
FAR14	Kirkwood	0.000
FAR14	Komatiepoort	0.000
FAR14	Louis Trichardt	0.000
FAR14	Lutzville	0.000
FAR14	Mt. Edgecombe	0.000
FAR14	Nelspruit	0.000
FAR14	Nkwalini	0.000
FAR14	Onrus River	0.024
FAR14	Paarl	0.039
FAR14	Pietermaritzburg	0.000
FAR14	Piketberg	0.000
FAR14	Pinetown	0.000
FAR14	Port Elizabeth	0.000
FAR14	Potchefstroom	0.000
FAR14	Pretoria	0.000
FAR14	Riebeeck Kasteel	0.000
FAR14	Rustenburg	0.037
FAR14	Somerset West	0.000
FAR14	Stellenbosch	0.038
FAR14	Tzaneen	0.039

FAR15	Bloemfontein	0.000
FAR15	King Williams Town	0.000
FAR15	Kirkwood	0.000
FAR15	Komatiepoort	0.070
FAR15	Louis Trichardt	0.027
FAR15	Lutzville	0.000
FAR15	Mt. Edgecombe	0.198
FAR15	Nelspruit	0.000
FAR15	Nkwalini	0.000
FAR15	Onrus River	0.114
FAR15	Paarl	0.000
FAR15	Pietermaritzburg	0.000
FAR15	Piketberg	0.000
FAR15	Pinetown	0.162
FAR15	Port Elizabeth	0.000
FAR15	Potchefstroom	0.088
FAR15	Pretoria	0.036
FAR15	Riebeeck Kasteel	0.000
FAR15	Rustenburg	0.043
FAR15	Somerset West	0.019
FAR15	Stellenbosch	0.000
FAR15	Tzaneen	0.024
FAR16	Bloemfontein	0.084
FAR16	King Williams Town	0.071
FAR16	Kirkwood	0.025
FAR16	Komatiepoort	0.000
FAR16	Louis Trichardt	0.048
FAR16	Lutzville	0.033
FAR16	Mt. Edgecombe	0.000
FAR16	Nelspruit	0.055
FAR16	Nkwalini	0.000
FAR16	Onrus River	0.000
FAR16	Paarl	0.006
FAR16	Pietermaritzburg	0.148
FAR16	Piketberg	0.069
FAR16	Pinetown	0.136
FAR16	Port Elizabeth	0.005
FAR16	Potchefstroom	0.000
FAR16	Pretoria	0.000
FAR16	Riebeeck Kasteel	0.194
FAR16	Rustenburg	0.070
FAR16	Somerset West	0.000
FAR16	Stellenbosch	0.038
FAR16	Tzaneen	0.037